HEPATO PROTECTIVE AND ANTIOXIDANT ACTIVITY OF *CNIDOSCOLUS PHYLLACANTHUS* AGAINST D-GALACTOSAMINE INDUCED OXIDATIVE STRESS IN RATS

THESIS

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MASTER OF PHARMACY
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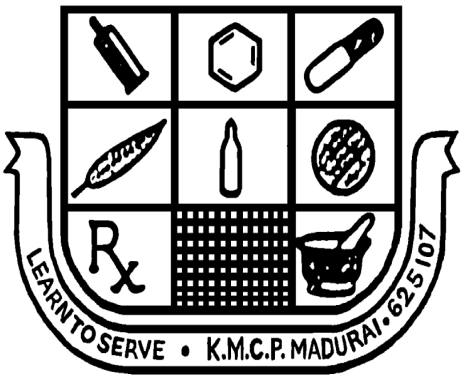

DEPARTMENT OF PHARMACOLOGY
K.M. COLLEGE OF PHARMACY
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Dedicated to Almighty

And My Beloved

family& Friends

CONTENTS

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The secret of success is undaunted ardor, motivation, dedication, confidence on self and above all the blessing of God. I bow in reverence to the Almighty for bestowing upon me all his kindness that has helped me throughout the journey of my life. Success is an outcome of collaborated efforts aimed that achieving different goals. I hereby take this opportunity to acknowledge all those who have helped me in the completion of this dissertation work.

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Thank you!!!!

Dr. D. Stephen,The American College,
Lecturer
Department of Botany **Madurai-2**

CERTIFICATE

This is to certify that the plant specimen brought to me by **Mr. A. RAJASEKARAN,** II year **M.Pharm (Pharmacology); Student of K.M. College of pharmacy,** Madurai has been identified as **cnidoscolusphyllacanthus**belonging to the family**euphorbiaceae.**

Dr.D.Stephen.

Date : 22/08/2013
Madurai
Tamil nadu

THE AMERICAN COLLEGE MADURAI DEPARTMENT OF BOTANY

In a sense , there has always magic in plants , an unknown Genie , mysterious and omnipotent, an all pervading powerful force. From the time , man first started looking for medicine to cure illness or combination for potential products for magic remedies of unconquerable and almost fatal ailments , plants and herbs have continuously reminded mysterious to him .

Plants have been utilised as a natural source of medicinal compounds since thousands of years. Human is using numerous plants and plant derived products to cures and relief from various physical and mental illness. These plants are used in traditional Chinese, Ayurveda, Siddha, Unani and Tibetan medicines. Ancient literature such as Rigveda, Yajurveda, Atharvaveda, CharakSamhita and SushrutSamhita also describes the use of plants for the treatment of various health problems. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems.

In last five decades, these plants have been extensively studied by advanced scientific techniques and reported for various medicinal properties viz, anticancer activity, antibacterial activity, antifungal activity, antidiabetic activity, antioxidant activity, hepatoprotective activity, haemolytic activity, larvicidal activity and anti-inflammatory activity etc.[(1)]

The liver is a vital organ of involved in the metabolism of nutrients such as carbohydrates, proteins and lipids and excretion of waste metabolites and detoxification of the exogenous and endogenous challenges like xenobiotics, drugs, viral infections and chronic alcoholism. A liver disease is a worldwide problem; conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. Herbal drugs have gained importance and popularity in recent years is numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practice as well as traditional system of medicine in India.

Many naturally occurring products have been reported to contain large amount of antioxidant other than vitamin C, E and carotenoid. These antioxidant play a vital role in delaying, intercepting or preventing oxidative reactions, catalyse by free radical[(2)]

In this present study I have selected a plant , which has shown good hepatoprotective as well as antioxidant properties. Undoubtedly , the plant kingdom still hold many species of plant containing substances of medicinal value, which

have yet to be discovered ; large numbers of plants are constantly being screened for their possible pharmacological value particularly for their hepatoprotective properties.

FREE RADICALS

Since humans or human ancestors first evolved, adestructive class of chemical agents has assailed thehuman body. They are called “free radicals”, though theyare also termed “reactive oxygen species” andabbreviated to “ROS”. They assailed even our preprimateancestors. They assailed the dinosaurs and allother life forms that exist in the fossil record. Even thesimplest single-celled organisms that have an oxidativemetabolism are and always have been assailed by thesesame free radicals. The free radicals come from oxygenand highly oxygenated molecules.[3]

Free radicals are atoms or molecules containing unpaired electrons. Electrons normally exist in pairs in specific orbitals in atoms or molecules. Free radicals, which contain only a single electron in such any orbital, are usually unstable toward losing or picking up an extra electron, so that all electrons in the atom or molecule will be paired. Free radicals can be positively charged, negatively charged, or neutral. The presence of an unpaired electron in an atom or molecule provides great reactivity, thus shortening its half-life.[4]

Free radicals are commonly generated via NADPH cytochrome P-450 reductase or other flavin containing reductases,although cytochrome P-450 itself may involved ,as is the case in the reduction of carbon tetrachloride to form radicals $\cdot CCl_3$ and $\cdot CCl_2O_2$.Many radical can participate in recycling reaction, resulting in a sustained level of free radicle in the cell, result in depletion of reduced cofactor and hypoxia[5]

TYPES OF FREE RADICALS

Most free radicals are coming from oxygen atoms and are called Reactive Oxygen Species (ROS), such as superoxide ion, hydroxyl radical, hydrogen peroxide and singlet oxygen.

Superoxide ion (or reactive oxygen species)

It is an oxygen molecule with an extra electron. This free radical can cause damage to mitochondria, DNA and other molecules. Our body can neutralize superoxide ions by producing superoxide dismutase.

Hydroxyl radical

It is formed by the reduction of an oxygen molecule in the electron transport chain. It is a neutral (not charged) form of the hydroxide ion. Hydroxyl radicals are highly reactive and form an important part of radical biochemistry. Unlike superoxide the hydroxyl radical cannot be eliminated by an enzymatic reaction. Is has a very short half-life and will only react with molecules its vicinity. Because of its high reactivity it will damage most organic molecules such as carbohydrates, DNA, lipids and proteins.

Singlet oxygen

Singlet oxygen is formed by our immune system. Singlet oxygen causes oxidation of LDL cholesterol.

Hydrogen peroxide

It is not a free radical but it is involved in the production of many reactive oxygen species. Hydrogen peroxide is a by-product of oxygen metabolism and is neutralized by peroxidases .

Sometimes reactive nitrogen atoms are involved and these free radicals grouped under Reactive Nitrogen Species (RNS). Nitric acid is the most important RNS. Some transitional metals, such as iron and copper, have many numbers of unpaired electrons and can also act as free radicals. These metals do not have that strong electron affinity but can easily accept and donate electrons. [6]

GENERATION OF FREE RADICAL

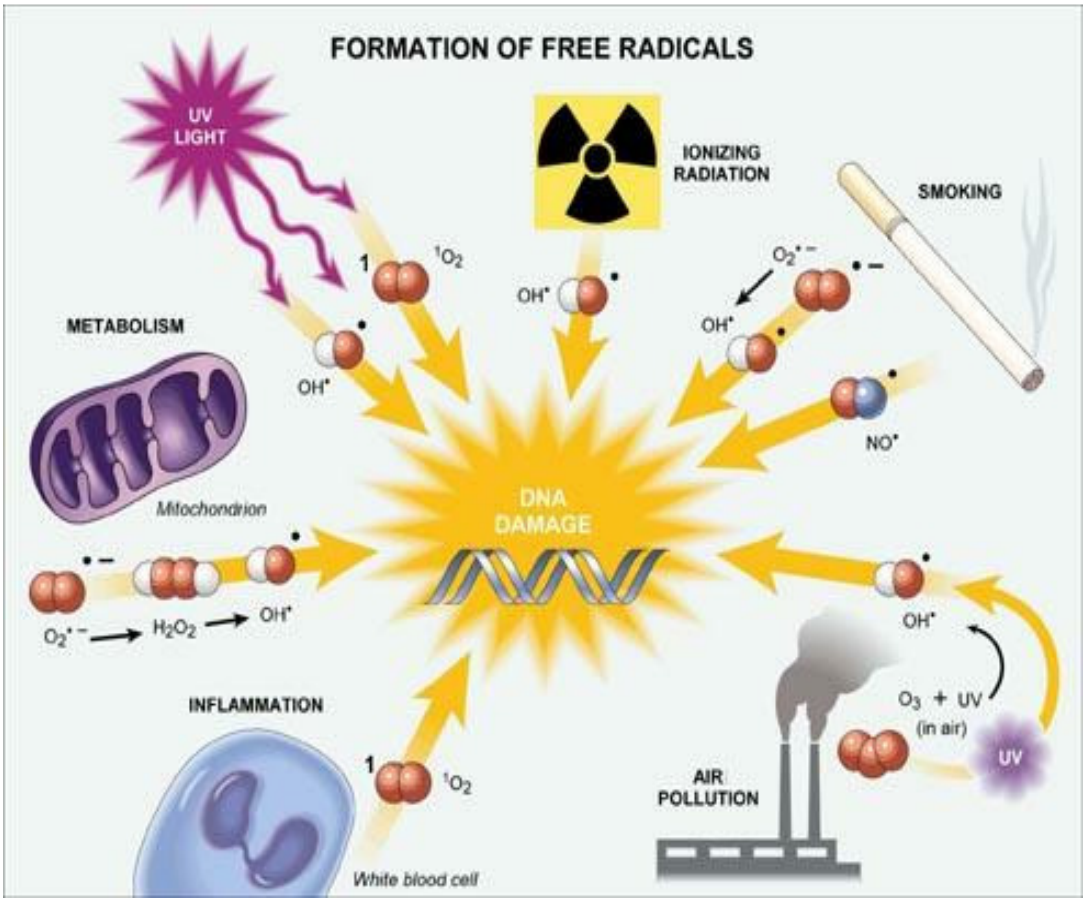
Many chemical compounds can be converted into free radicalforms. The latter species are usually quite reactive and short lived because they have a single unpaired electron in theirouter orbital. Recently, evidence has accumulated to indicatethat free radical intermediates may be important in the toxicityof a large number of substances. Free radicals can beformed by several mechanisms, including one-electron oxidation,one-

electron reduction, homolytic cleavage of a C-Hbond, and in some cases by two-electron oxidation/reductionreactions. Enzyme-catalyzed oxidation also provides a mechanismby which free radical intermediates can be formed.[7]

Oxidative stress may be defined as an imbalance between pro-oxidant and antioxidant agents, in favour of the former, this imbalance may be due to an excess of pro-oxidant agents, a deficiency of antioxidant agents or both factors simultaneously. The origin of oxidative stress is an alteration of the redox status in cells, leading to a cellular response to counteract the oxidising action.

Pro-oxidant agents are all those that can directly or indirectly oxidise molecules. The most important pro-oxidant agents in biological systems are those derived from oxygen, more commonly known as reactive oxygen species (ROS), although there are also reactive species derived from nitrogen (RNS) or sulphur (RSS). Some of these molecules exhibit great reactivity, such as hydroxyl radicals (HO.), and others present mild reactivity. The biological importance of the latter relies on their capacity to be easily transformed into the hydroxyl radical, especially in the presence of iron, as in the case of superoxide radicals (O_2) or hydrogen peroxide (H_2O_2).

The production of these reactive species occurs continuously in the organism; this production may be endogenous or exogenous. Some of these reactive species are generated as “chemical accidents”, i.e. undesired secondary reactions between biomolecules or alternatively in the detoxification of xenobiotic. Other reactive species, however, are generated in vivo for a specific aim such as in the case of activated phagocytes which produce O_2, and H_2O_2.[8]



Free radical formation

(Fig.no:1)

STEPS INVOLVING FREE RADICAL GENERATION

In chemistry, free radicals take part in radical addition and radical substitution as reactive intermediates. Chain reactions involving free radicals can usually be divided into three distinct processes: initiation, propagation, and termination.

Initiation reactions are those, which result in a net increase in the number of free radicals. They may involve the formation of free radicals from stable species or they may involve reactions of free radicals with stable species to form more free radicals.

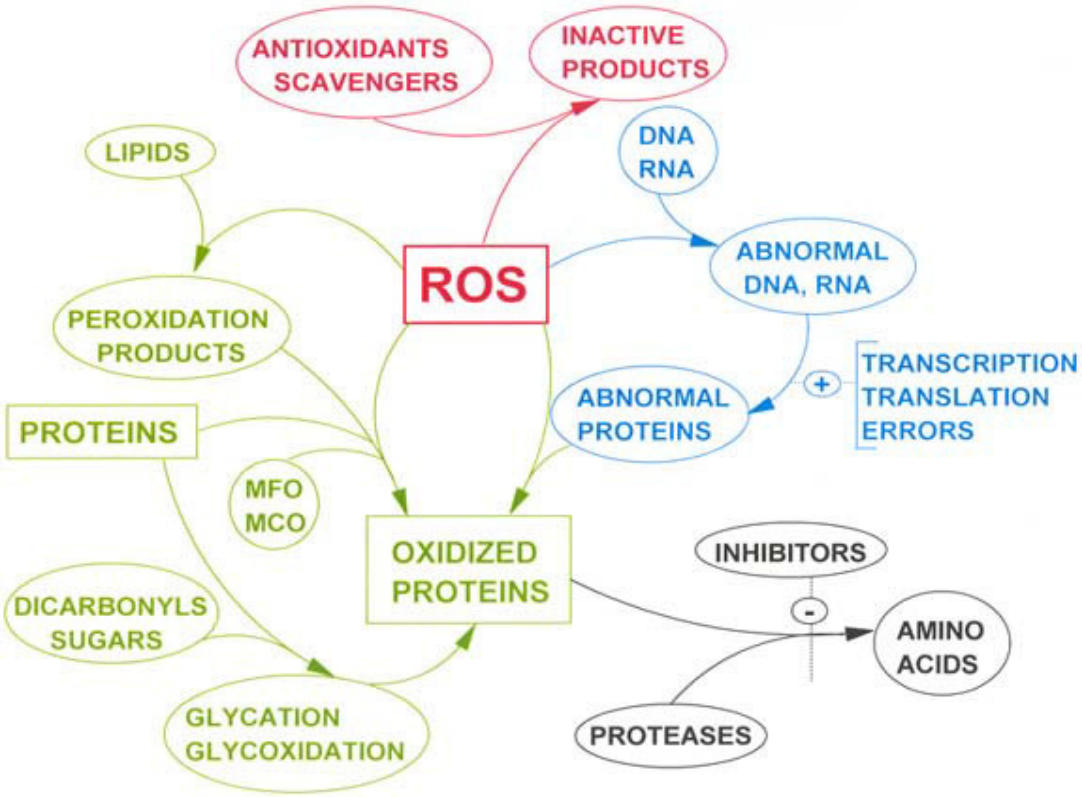
Propagation reactions involve free radicals in which the total number of free radicals remains the same.

Termination reactions are those reactions resulting in a net decrease in the number of free radicals. Typically two free radicals combine to form a more stable species, for example: $2Cl\cdot \rightarrow Cl_2$

The formation of radicals may involve breaking of covalent bonds homolytically, a process that requires significant amounts of energy. The bond energy between two covalently bonded atoms is affected by the structure of the molecule. Homolytic bond cleavage most often happens between two atoms of similar electronegativity. However, propagation is a very exothermic reaction. Radicals may also be formed by single electron oxidation or reduction of an atom or molecule. An example is the production of superoxide by the electron transport chain.[7]

Reactive oxygen species (ROS)

Reactive oxygen species (ROS) are very small molecules and are highly reactive due to the presence of unpaired valence shell electrons. ROS is formed as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling. However, during times of environmental stress ROS levels can increase dramatically, which can result in significant damage to cell structures. Platelets involved in wound repair and blood homeostasis release ROS to recruit additional platelets to sites of injury. Generally, harmful effects of reactive oxygen species on the cell are most often like - Damage of DNA, oxidations of polydesaturated fatty acids in lipids, oxidations of amino acids in proteins, oxidatively inactivates specific enzymes by oxidation of co-factors. The effect of ROS can be simply explained by the following fig.no:2.[9]



Effects of ROS (fig:2)

SOURCES OF REACTIVE FREE RADICALS

a) Mitochondrial Cytochrome Oxidases

The free radicals are formed continuously as normal by products of cellular metabolism under normal conditions, about 95% of molecular oxygen in biological system undergoes controlled reduction through the addition of four electrons in the mitochondrial cytochrome oxidase system to form water. The remaining molecular oxygen leaks from this pathway and undergoes sequential univalent reduction to produce superoxide anion (O_2), hydrogen peroxide(H_2O_2) and highly reactive hydroxyl radical(OH).[10]

b) Purine Metabolism

The enzyme xanthine dehydrogenase (XDH) mainly located in the vessel walls of most tissue including cardiac and skeletal muscle, catalyses the oxidation of hypoxanthine

to xanthine and xanthine to uric acid. Under certain conditions like Ischemia reperfusion and extreme hypotension as in hemorrhagic shock xanthine dehydrogenase may be either reversibly or irreversibly transformed to xanthine oxidase (XO). In contrast to xanthine dehydrogenase, xanthine oxidase utilizes the oxygen as electron acceptor and produces super oxide as a result while catalysing the oxidation of hypoxanthine to uric acid.[11]

c) Phagocytes

As weapons for pathogen destruction and immune protection ROS have been put togood use by phagocytes NADPH oxidase located in the plasma membrane of neutrophils produce superoxide's on purpose following spontaneous dismutation superoxide's generated as such remarkably contribute to H_2O_2 formation, cytoplasmic azurophilic granules of neutrophils and to a lesser extent monocytes contain a heamo proteins peroxidase called myeloperoxidase when activated by immune challenge or such other stimuli, neutrophils release myeloperoxidase in to the extra cellular medium[12]. The released myeloperoxidase complexes with H_2O_2 to form an enzyme substrate complex with an oxidising potential. The complex oxidises chloride(Cl^-) to produce hypochlorous acid (HOCl).[13]

d) Drug metabolism

Microsomal and nuclear membrane electron transport system, mainly involved in drug metabolism (via cytochrome P_{450} and B_5 systems) also host ROS production NADPH oxidation both in presence and absence of mixed function oxidase substrates contribute to ROS (O_2 and H_2O_2) formation as well. Mechanism of cytochrome P450 driven reactions involves the formation of oxy and subsequently peroxyintermediates[14]. Breakdown of these intermediates yield ROS. Cytochrome P450 has functional multiplicity and also acts as peroxidase in which peroxidases are used as oxygen donor[15].

e) Nitric Oxide synthase

It is widely believed that endothelium derived relaxing factor (EDRF) produced by vascular endothelial cells is identical with NO (Nitric oxide)[16]. Nitric oxide is synthesised in a wide variety of tissues and is known to be implicated in a number of crucial physiological functions eg: control of systemic blood pressure, respiration, digestion, platelet aggregation etc. The enzyme primarily responsible for the synthesis of NO is tissue specific. Nitricoxide synthase in the endothelium and neurons is calmodulinactivated

enzyme that oxidises Arginine to Citrulline in the presence of biopterin, NADPH and oxygen. Cells like macrophages which are capable of producing both NO and super oxides are the likely host of a very powerful deleterious ROS the peroxy nitrite anion (ONOO). This formed peroxy nitrate anion is relatively long lived ROS and the NO may magnify superoxide toxicity remarkably[17].

f) Transition Metals

Conditions (eg: Plasma pH < 6, haemolysis and ischemia- reperfusion) that lead to the release of transition metal ions (that of Iron and Copper) may remarkably amplify ROS toxicity[18]. Iron and Copper ions are capable of converting H_2O_2 to OH. In the presence of the free transition metal ions ascorbic acid, a commonly known antioxidant functions as a pro oxidant[19].

g) Some other possible sources

There are some other enzymes known to be responsible for the generation of free radicals are listed below with their respective sub cellular localisation[20].

- Glycolate oxidase (Peroxisome)
- L-alpha hydroxyl acid oxidase (Peroxisome)
- L-gulonlactone oxidase (Cytosol)
- Aldehyde oxidase(Cytosol)
- Monoamino oxidase (Mitochondrial outer membrane)
- Urate oxidase (Peroxisome core)
- Diamine oxidase (Endoplasmic reticulum)[21]

PGH(Prostagandin)synthase dependent arachidonic acid metabolism generates superoxide's in the presence of NADH.Once other possible biological source of ROS is myoglobin oxidation of ferrous myoglobin to its hypervalentferryl form is suggested to contribute to ischemia reperfusion injuary in the heart.[22]

TARGETS OF FREE RADICAL IN VIVO

Free radicals attack three main cellular components.

Lipids

Lipid peroxidation is the introduction of a functional group containing twocatenated oxygen atoms, O-O, into unsaturated fatty acids in a free radical reaction Polyunsaturated fatty acids susceptible to free radical attack are initiated by the formation of a carbon-centered radical by the abstraction of a hydrogen atom at one of the double bonds of the lipid. Lipid peroxidation is also one of major causes of quality deterioration during the storage of fats, oils or other lipid-rich foods. Lipid peroxidation is the most extensively studied manifestation of oxygen activation in biology. It is broadly defined as "oxidative deterioration of PUFA" which are fatty acids that contain more than two carbon carbon double bonds. Lipids when reacted with free radicals can undergo the highly damaging chain reaction effects Peroxidation of lipids in cell membranes can damage cell membranes by disrupting fluidity and permeability. Lipid peroxidation can also adversely affect the function of membrane bound proteins such as enzymes and receptors.

Proteins

Oxidative damage to proteins can be caused by free radicals. During the mitochondrial electron transport chain, freeradicals are produced which can stimulate protein degradation. Oxidative protein damage may be brought about by metabolic processes which degraded damaged protein to promote synthesis of a new protein. The mechanism of oxidative damage of proteins by ROS has been studied invitro by generating these reactive species either in solution or site specifically within the protein. While the former damage is termed as nonspecific (global) and the later damage is termed as site specific (localised damage). Nonspecific damage can be stimulated by generating activated oxygen species in-situ, using either a radiation source, CO, or using pulse radiolysis techniques which lead to aggregation and fragmentation of the protein and modification of almost all the amino acids.[23]

DNA

Fragmentation of DNA caused by free radical attack causes activation of the poly (ADP-ribose) synthetase enzyme. This splits NAD+ to aid the repair of DNA. However, if

the damage is extensive, NAD+ levels may become depleted to the extent that the cell may no longer be able to function and dies. The site of tissue damage by free radicals is dependent on the tissue and the reactive species involved. Extensive damage can lead to death of the cell; this may be by necrosis or apoptosis depending on the type of cellular damage. When a cell membrane or an organelle membrane is damaged by free radicals, it loses its protective properties. This puts the health of the entire cell at risk.[9]

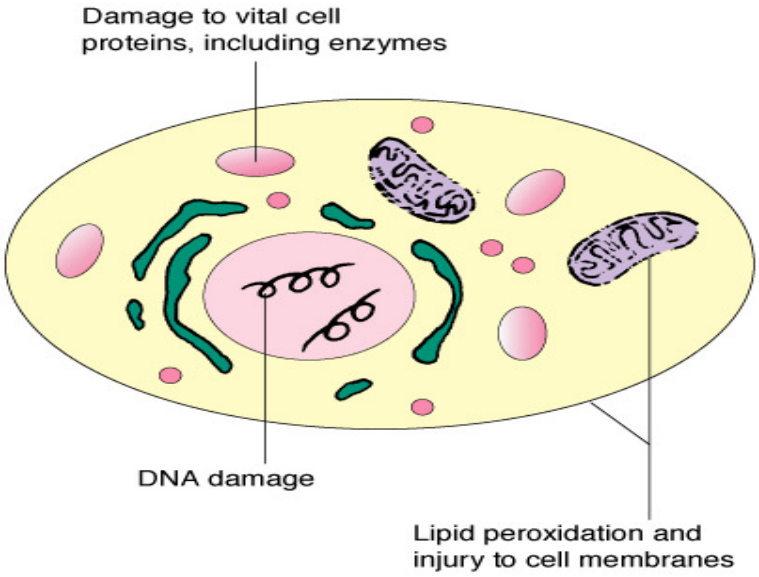


Fig.no.3

FREE RADICAL REACTIONS IN DNA STRAND BREAKAGE

(i) Formation of Oxygen Radicals

Oxygen radicals are usually formed via redox reactions because oxygen is the most efficient biological electron acceptor forms the superoxide radical $O_2^{-\cdot}$

$$R\text{-}X^{-\cdot} + O_2 \rightarrow R\text{-}X + O_2^{-\cdot}$$

Superoxide radical can undergo dismutation to hydrogen peroxide and oxygen.

$$2\ O_2^{-\cdot} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

$$2H_2O_2 \xrightarrow{CATALASE} 2H_2O + O_2$$

The hydrogen peroxide can be removed by catalase or glutathione peroxidase. However, if a trasition metal is present, commonly Fe or Cu, the metal can be reduced and hydroxyl radical formed ($OH^{\cdot}$).

$$H_2O_2 + 2GSH \xrightarrow{\text{glutathione peroxidase}} GSSG + H_2O$$

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^{\cdot} + OH^{-} \text{ (fentons reaction)}$$

$$O_2^{-\cdot} + Fe^{3+} \rightarrow Fe^{2+} + O_2$$

Heber Weiss reaction

Summarised equation

$$O_2^{-\cdot} + H_2O_2 \rightarrow OH^{\cdot} + OH^{-} + O_2$$

There is some evidence that free radical damage contributes to the etiology of many chronic health problems such as emphysema, cardiovascular and inflammatory diseases, cataracts, and cancer. Defences against free radical damage include tocopherol (vitamin E), ascorbic acid (vitamin C), beta-carotene, glutathione, uric acid, bilirubin, and several metalloenzymes including glutathione peroxidase (selenium), catalase (iron), and superoxide dismutase (copper, zinc, manganese) and proteins such as ceruloplasmin (copper). The extent of tissue damage is the result of the balance between the free radicals generated and the antioxidant protective defence system. Several dietary micronutrients contribute greatly to the protective system.

(ii) Radical induced DNA damage

Hydroxyl radical formed by radiolysis or by drug induced mechanisms, interact with DNA bases to form a hydroxyl radical adduct. It appears that Thymidine and to a lesser extent, Adinine may be rather more sensitive bases for hydroxyl radical attack than others, but in any event a neutral hydroxyl radical formed. If an electron is now removed from the neutral hydroxyl base radical to form a positively charged base adduct, the damage is fixed, that is can no longer be repaired. In well oxygenated cells this process is easily accomplished by oxygen which accepts an electron to form superoxide. At this stage hydroxyl base cation adduct can undergo 2 chemical transformation: loss of a proton to

form a hydroxyl base, or a condensation reaction in which a second hydroxyl group is added to the base to form a glycol derivative of the base which is rapidly recognised as aberrant by the cell's repair enzymes and the base is efficiently removed. The result of the damaged base is strand breakage, the extent of which, if unable to repaired, will result in cytotoxicity, possible mutagenicity and /or carcinogenicity and/or cell death[24].

(iii) Redox cyclic.

If a drug required reductive activation to produce its damaging effect upon the cell, this reaction may be nullified in the presence of oxygen which is the most electron affinic of all biological molecules. The overall effect is one of redox cyclic, which if it prevents the drug from being reductively activated, is also called futile cyclic.

Eg. Nitro compound which upon being reduced by a singlet electron, forms a nitro radical anion. Oxygen being more electron affinic than the nitro radical, abstracts the electron, regenerating the parent drug molecule to form superoxide. The formed hydroxyl radical sis not only causing DNA damage but also in causing the peroxidation of lipid in membranes or oxidation of amino acids in proteins leading to conformational changes and inactivation of enzymes.

$R\text{-}NO_2 + 1e^- \rightarrow R\text{-}NO_2^{-\cdot} + O_2 \rightarrow R\text{-}NO_2 + O_2^{-\cdot}$

(iv) Action of nitro homocyclic and hetrocyclic drugs

With all nitro compounds their activity is solely dependent upon reduction of the nitro group, the products of which are responsible for DNA damage. [25]

ANTIOXIDANT

Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentrationand thus have diverse physiological role in the body. Antioxidant constituents of the plantmaterial act as radical scavengers, and helps in converting the radicals to less reactive species. Avariety of free radical scavenging antioxidants is found in dietary sources like fruits, vegetablesand tea, etc.Antioxidants that have traditionally been used to inhibit oxidation in foods also quench dreadedfree radicals and stop oxidation chains *in-vivo*, so they have become viewed by many as nature'sanswer to environmental and physiological stress, aging, atherosclerosis, and cancer. Thenutraceutical trend towards doubling the impact of natural antioxidants that stabilize food andmaximize health impact

presents distinct challenges in evaluating antioxidant activity of purifiedindividual compounds, mixed extracts, and endogenous food matrices and optimizingapplications.[26]

Broadly the possible mechanisms by which antioxidants may protect against ROS toxicity are

1) Prevention of ROS formation
2) Interception of ROS attack by scavenging the reactive metabolites and converting them to less reactive molecule and by enhancing the resistivity of sensitive biological targets to ROS attack
3) Facilitating the repair of damage caused by ROS
4) Providing(e.g as a cofactor by acting to maintain a suitable redox status) a favourable environment for the effective function of other antioxidant[27]

In human body, a complex combination of enzymatic and no enzymatic functions to minimize the stress induced by ROS. These antioxidants can be classified as:

1) Endogenous antioxidant –those which are physiological in origin
2) Exogenous antioxidant- those which cannot be produced by the human body but may protect against pro-oxidant forces when administered as supplement[28,29]

ENDOGENOUS ANTIOXIDANT

Although a large number of enzymatic and nonenzymaticphysiological substances are known to have antioxidant like functions the primary contributors are;

a) superoxide dismutase's(SOD)

These are the enzymes involved in the cellular defence against uncontrolled oxidative process that catalyse the dismutation of the superoxide radical anion and hence diminishes toxic effect due to this or to other free radicals derived from secondary reactions.[30]

In mammalian tissue two types of SOD have been described;

- cystosoliccuprozinc- SOD(CuZn-SOD)
- mitochondrial mangano-SOD(Mn-SOD)

The principal function of Sod is to catalyse the conversion of one form of ROS to the other

The biosynthesis of SOD is mainly controlled by its substrate the O_2

$2O_2 + 2H \xrightarrow{SOD} H_2O_2 + O_2$

H_2O_2 thus produced is detoxified either by catalase or reduced glutathione (GSH) dependent reaction[31]

b) Catalase

Catalases are present virtually in all mammalian cells and are suggested to play a dual role

1) A true catalytic role in the decomposition of H_2O_2

   $H_2O_2 \xrightarrow{catalase} H_2O_2 + o_2$

2) A peroxidic role in which the peroxide is utilized to oxidize a range of H donors (AH_2) such as methanol, ethanol and formate

   $AH_2 + H_2O_2 \rightarrow A + 2\ H_2O_2$

In each case active enzyme- H2O2 complex is formed initially followed by an exceedingly rapid second stage in which a second molecule of H_2O_2 serves as an H donor for the enzyme H_2O_2 complex.The enzyme is most localized in the peroxisomes (mitochondria) of liver & kidney &in much smaller aggregates (microperoxisomes) found in other cells.[32]

c) Glutathione(L-Gamma-glutyl-L-cysteinyl glycine)

It is important in the circumvention of oxidative stress detoxification of electrophile maintenance of intracellular thiol redox status.[33]

Glutathione peroxidase (GSHPx) - Glutathione peroxidase catalase the reaction of hydroperoxide with reduced glutathione (GSH) to form glutathione disulphide (GSSG) and the reduction product of hydroperoxide. This enzyme is specific for its hydrogen donor GSH and nonspecific for the hydroperoxide ranging from H_2O_2 to organic hydroperoxide. Two third of the enzyme is present in the cytosol and one third in the mitochondria

$ROOH/H_2O_2 \xrightarrow{GSH\ Peroxidase} ROH/H_2O + GSSG$

The cystolic and membrane bound monomer GSH phospholipids hydroperoxidesGSHPx and the distinct tetramer plasma GSHPx is able to reduce phospholipids hydroperoxides without the necessity of prior hydrolysis by phospholipase A2. Glutathione S-transferase catalyses the reaction between the SH group of GSH and

potential alkylating agents there by neutralizing their electrophilic sites and rendering them more water soluble. GSH also play a central role in coordinating the synergism of various crucial antioxidants.[34,35]

d) Heme peroxidase

Heme peroxidase such as horseradish peroxidase, lacto peroxidase and other mammalian peroxidases. The enzyme catalyses the oxidation of a wide variety of electrons donors with the help of H_2O_2 and thereby scavenges the endogenous H_2O_2.[36]

$H_2O_2 + AH_2 \rightarrow 2\ H_2O_2 + A$

EXOGENOUS ANTIOXIDANTS

For the effective protection against oxidative insults that we encounter in our daily lives regular consumption of at least some antioxidants in the diet or as supplements, appears to be very crucial. Among the exogenous antioxidantsvitC&vitE have been recognised to be especially important and deficiency of these may leads to a number of pathophysiological consequences.

Vitamin C and Citrus Bioflavonoids

Vitamin C (ascorbic acid) exerts an antioxidant effect by undergoing oxidation to dehydroascorbic acid and then being regenerated. Because ascorbic acid is being constantly regenerated there is always fresh ascorbic acid available to continue the work of oxygen quenching mainly O_2 ,OH,and various lipid peroxides. The deficiency disease associated with Vitamin C is scurvy. Many of its symptoms reflect difficulty in forming new good quality connective tissues. However, it has been found consistently that Vitamin C acts best in the present of plant bioflavonoids and as a result Vitamin C is often mixed with citrus bioflavonoidspriorto being formed into supplement products. However special attention should be given in the presence of Fe^{3+} or Cu^{2+} excess. Vit C may acts as a strong pro-oxidant and may actually induce lipid peroxidation and oxidative modification of oxidative of genomic structure. Under such condition Vit C may reduce Fe^{3+} to Fe^{2+} which in turn facilitate the generation of OH.[37]

Vitamin E

The principal action of Vitamin E is now recognised to be the protection of the phospholipids of the cell membranes from free radical attack. This includes not only the outer cell membrane but also the much larger area of the internal cell membranes. Vitamin E is only one of several antioxidant nutrients within the cell, but the special connection between Vitamin E and membranes is assured by the fact that Vitamin E is both fat soluble and hydrophobic and that it also readily finds a location within the membranes between the assembled phospholipid molecules there. Vitamin E is therefore the antioxidant that is in situ within the membranes ready to deal with free radicals that arise within that exact location. Vitamin E appears a key factor in our overall antioxidant defence, but also to be especially significant in the nervous system[38].

Beta-Carotene

Beta-carotene is at the same time a Vitamin precursor thatthe body uses to make vitamin A, a carotenoid and aphytonutrient. It greatlyenhances the immune system. It is a powerful antioxidant andfree radical scavenger. Beta-carotene is the most efficientneutralizer of singlet oxygen, which has particularly high energy,and is one of the most destructive ROS molecules.

Alpha-carotene

Although, among the carotenoids, beta-carotene is afocus of attention for the supplement industry, mostresearch studies show alpha-carotene to be more potentas an antioxidant. Outstandingly, alpha-carotene wasfound to be ten times more potent as an anti-canceragent than beta-carotene and38% more potent as an antioxidant than beta-carotene. It seems wise, therefore, to include thealpha form into the best quality antioxidant formulae.

Lycopene & Lutein

Lycopene is another carotenoid antioxidant and is evenmore powerful than alpha-carotene. Lycopene and lutein in small doses may potentially preventcolon carcinogenesis. Lutein was shown to be important inprevention of lung cancer. The carotenoid antioxidants have also been found to beespecially important in the natural protection of the

eyeagainst macular degeneration. Lutein, zeaxanthinandlycopene have been found to bestow a good level ofprotection.

Minerals

The minerals required for forming the superoxide dismutaseenzymes do not have to be part of the antioxidant mix. All thatis required is that good to generous dietary intakes of them bemaintained either by dietary care or by supplements. In fact,iron and copper may not need to be given and should neverbe given in excess. Excesses of either of these have beenreported to actually increase free radical generation by causingan imbalance between these minerals and Vitamin C.Magnesium is a special case. It could be included in anantioxidant strategy either alongside the antioxidants orotherwise so long as the subject's intake of it is fully adequate[39].

INTRACELLULAR CHANGES FOLLOWING THE OXIDATIVE STRESS

Oxidative stress induced cytotoxic effects appear to be mediated by a perturbation of intracellular free calcium (Ca^{2+}) and thiol homeostasis[40]. In a flow cytometric study it was observed that when skeletal muscle derived L-6 cells subjected to oxidative change, intracellular calcium sharply increased immediately following the challenges such as a response was followed by membrane disintegration as detected by propidium iodide staining of DNA[41]. An early response to oxidative stress is depletion (via oxidation or covalent adduct formation)of cellular soluble protein and bound thiol (eg.GSH). Such depletion can

1) Decrease plasma membrane Ca^{2+} ATPase activity and contribute to plasma membrane blabbing (altered permeability) and to impairment of the mitochondrial ability to retain Ca^{2+}.
2) Impair Ca^{2+} sequestration capacity of the endoplasmic reticulum(an organelle with high Ca^{2+} affinity in muscle playing a key role in fine tuning of cytosolic level of the cation) and
3) perturb microsomal Ca^{2+} homeostasis[42]

HEALTH AND DISEASE

Oxidative damage to DNA, proteins, and other macromolecules has been implicated in the pathogenesis of a wide variety of diseases, most notably heart disease and

cancer.A growing body of animal and epidemiological studies as well as clinical intervention trials suggest that antioxidants may play a pivotal role in preventing or slowing the progression of both heart disease and some forms of cancer.

Cardiovascular disease

ROS-induced oxidative stress plays a role in various cardiovascular diseases such as atherosclerosis, ischemic heart disease, hypertension, cardiomyopathies, cardiac hypertrophy and congestive heart failure major sources of oxidative stress in cardiovascular systeminvolve: (i) the enzymes xanthine oxidoreductase(XOR), (ii) NAD(P)H oxidase (multisubunitmembranecomplexes) and (iii) NOS as well as (iv) the mitochondrialcytochromes and (v) hemoglobin. NOSs and hemoglobinarealso principal sources of RNS, including NO• and SNOs, which convey NO• bioactivity.[43]

Ischemic/reperfusion injury

Ischemia-reperfusion injury is a clinically relevant problem occuring as damage to the myocardium following blood restoration after a critical period of coronary occlusion. Massive production of ROS during ischemia/reperfusionin turn lead to tissue injury causing thus serious complications in organ transplantation, stroke, and myocardial infarction. During ischemia, oversized ATP consumption leads to accumulation of the purine catabolites hypoxanthine and xanthine, which upon subsequent reperfusion and influx of oxygen are metabolized by xanthine oxidase to produce enormous amounts of superoxide radical and hydrogen peroxide.[44]

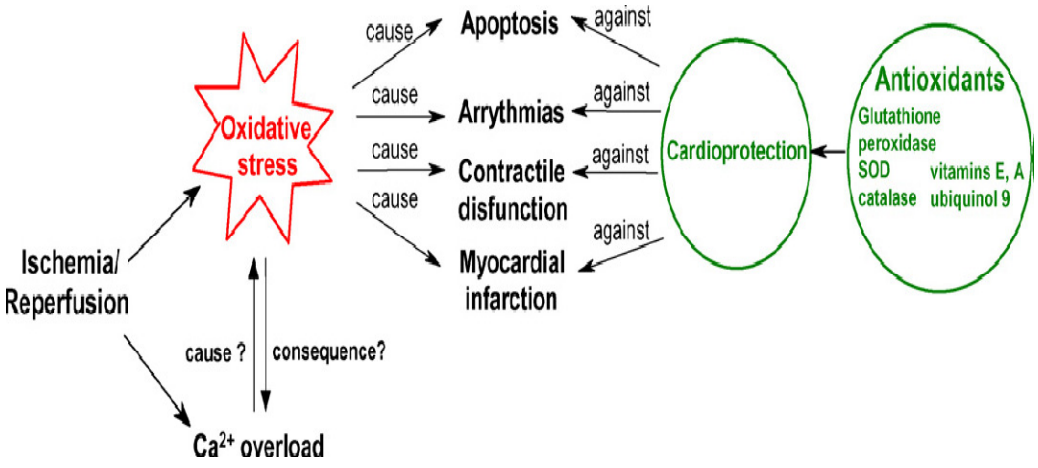


Fig.no.4: Effect of oxidative stress and antioxidants in pathophysiology of ischemia-reperfusion injury in the heart.

Rheumatoid arthritis

Rheumatoid arthritis is an autoimmune disease that causes chronic inflammation of the joints and tissuearound the joints with infiltration of macrophages and activated T cells. The pathogenesis of this disease is linked predominantly with the formation of free radicals at the site of inflammation. Oxidative injury and inflammatory status in variousrheumatic diseases was confirmed by increased levels of isoprostanes and prostaglandins in serum and synovial fluid compare to controls. Oxidative conditionsin synovial tissue are also associated with a higherincidence of p53 mutations.[45]

Diabetes

It is a condition in which there is a decreased uptake of glucose into muscle and adipose tissueleads to chronic extracellular hyperglycemia resulting in tissue damage and pathophysiological complications, involving heart disease, atherosclerosis, cataract formation, peripheral nerve damage, retinopathy and others. Increased oxidative stress has been proposed to be one of the major causes of the hyperglycemia-induced trigger of diabeticcomplications. Hyperglycemia in an organism stimulatesROS formation from a variety of sources. These sources include oxidative phosphorylation, glucose auto oxidation, NAD(P)H oxidase, lipooxygenase, cytochrome P450 monooxygenases, and nitric oxide synthase (NOS). Diabetic patients have been found to have higher levels of oxidative stress indices.Ithas been shown that under physiological condition glucose may undergo auto oxidation and contribute to ROS formation. ROS are capable of facilitating the glycation reaction that are now believed to be responsible for a most of the diabetic complication.[46]

Neurological disorders

The brain is particularly vulnerable to oxidative damage because of its high oxygen utilisation, its high content of oxidisable polyunsaturated fatty acids, and the presence of redox-active metals (Cu, Fe). Oxidative stress increases with age and therefore it can be considered as an important causative factor in several neurodegenerative diseases, typical for older individuals[47]

Alzheimer's disease

The brains of patients with Alzheimer's disease (AD)show a significant extent of oxidative damage associated with a marked accumulation of amyloid-β peptide (Aβ), the main constituent of senile plaques in brain, as well as deposition of neurofibrillary tangles

and neutrophil threadsThe direct evidence supporting increased oxidative stressin AD brain include (i) increased Cu, Fe, Al, and Hgcontent; (ii) increased lipid peroxidation and decreasedpolyunsaturated fatty acid content, and an increase in4-hydroxynonenal, an aldehyde product of lipid peroxidationin AD ventricular fluid; (iii) increased protein andDNA oxidation; (iv) diminished energy metabolism and decreased cytochrome *c* oxidase content; (v) advanced glycation end products (AGE), malondialdehyde, carbonyls, peroxynitrite, heme oxygenase-1, and SOD-1 in neurofibrillary tangles (vi) the presence in activated microglia surrounding most senile plaques of nitrotyrosine, formed from peroxynitrite(ONOO•−). The elevated production of Aβ, as apreventive antioxidant for brain lipoproteins under theaction of increased oxidative stress and neurotoxicityin ageing, is postulated to represent a major event inthe development of Alzheimer's disease.[48]

Parkinson's disease

Parkinson's disease (PD) involves a selective loss ofneurons in an area of the midbrain called the substantianigra. A majority of studies explored the effect of oxidativestress that contributes to the cascade of events leading to dopamine cell degeneration in PD . The occurrence of oxidative stress in PD is supported by both postmortem studies and by studies demonstrating the capacity of oxidative stress to induce nigral cell degeneration. There is evidence that there are high levels of basal oxidative stress in the substantianigraparscompacta (SNc) in the normal brain, but that this increases in PD patients. One of the earliest detectable changes in the PDbrain is a dramatic depletion in substantianigralevelsof the glutathione. Since oxidative stress appears to represent a portionof a cascade of biochemical changes leading to dopaminergicdeath, one of a major problem in understanding thepathogenesis of PD is separating out the effect and extentof oxidative stress from other components of the cascadethat themselves can play a primary role in the initiationof ROS and RNS[49].

Cancer

Epidemiological evidence consistently relates low antioxidant intake or low blood levels of antioxidants with increased cancer risk. Oxidants are capable of stimulating cell division, which is a critical factor in mutagenesis. When a cell with a damaged DNA strand divides, cell metabolism and duplication becomes deranged. Thus, a mutation can arise which in turn is an important factor in carcinogenesis. It is believed that antioxidants exert their protective effect by decreasing oxidative damage to DNA and by decreasing

abnormal increases in cell division. Although antioxidant activity is believed to be responsible for much of the protection against tumorgenesis, additional anticancer activities have been observed from several plant-derived substances. Sulfurcontaining phytochemicals, such as the allylsulfides found in the allium family (garlic, onions, and leeks), and isothyocyanates and sulphoraphane (cabbage, broccoli, and cauliflower) have been shown to inhibit various steps in tumor development in animal and in vitro studies. Indoles, also found in cruciferous vegetables, and terpenes, natural constituents of citrus oils, may also be protective.

PULMONARY DISORDER

Because of its large surface area, the respiratory tract is a major target for free radical insult, not to mention the fact that air pollutionis a major source of ROS. Recent studies suggest that freeradicals may be involved in the development of pulmonary disorderssuch as asthma. Cellular damage caused by free radicals isthought to be partly responsible for the bronchial inflammationcharacteristic of this disease. It has been suggested that increasingantioxidant intake may help to reduce oxidant stress and help toprevent or minimize the development of asthmatic symptoms. Vitamin C, vitamin E, and beta carotene supplementation has beenassociated with improved pulmonary function. Some evidencesuggests glutathione, or possibly N-acetyl cysteine, which is aprecursor to glutathione, may be helpful in protecting againstpulmonary damage as well.[50]

Fibrosis:

Oxygen, paraquat, nitrofurantoins, and bleomycin, produces pulmonary fibrosis. Radical-generating agents such as iron and copper are also associated with liver fibrosis (cirrhosis) and fibrotic changes in other organs such as the heart. The induction of vitreous scarring by interocular iron or copper is also well known, as is the association of homocystinuria with fibrotic lesions of the arteries. Adult Respiratory Distress Syndrome (ARDS) occurs due to production of active oxygen species by inflammatory cells.

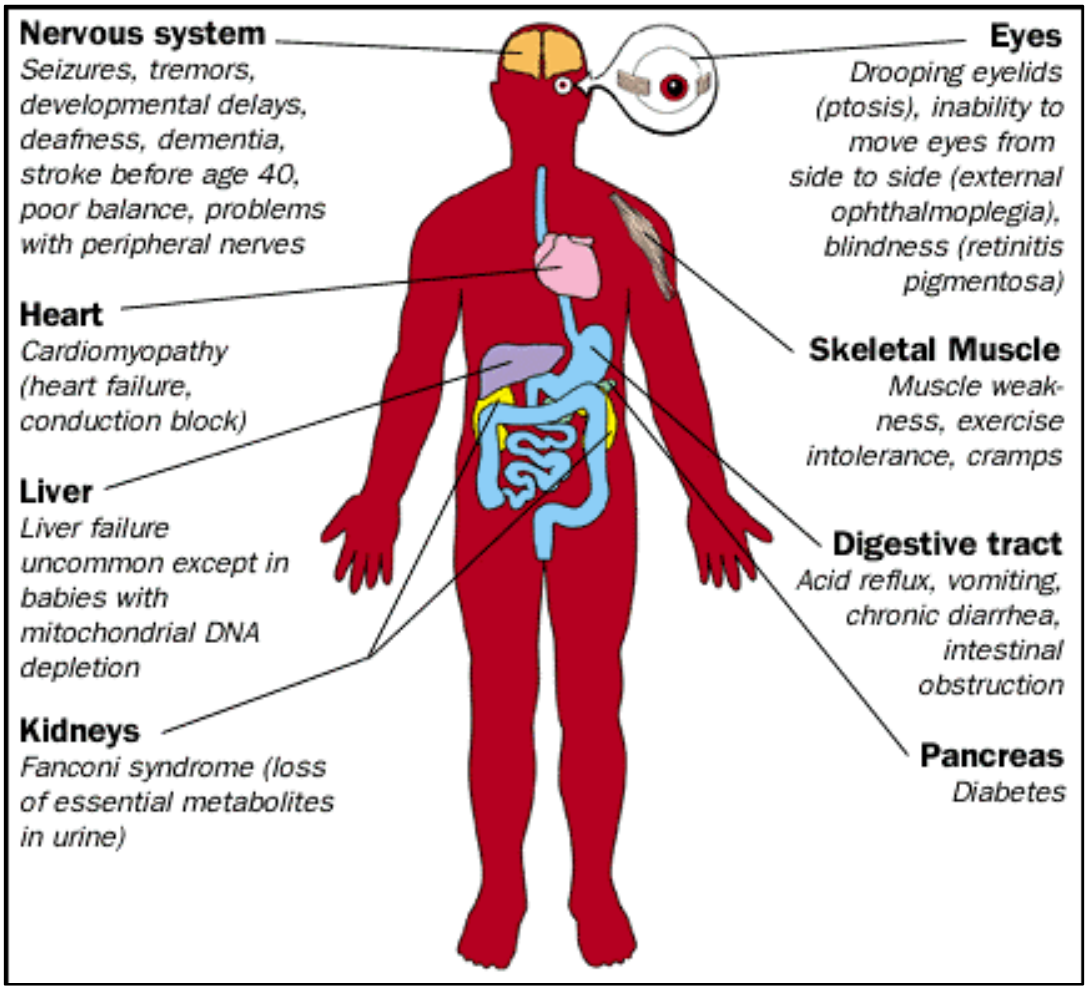


Fig.no.5

LIVER

Liver is the largest and the only human internalorgan that actually can regenerate itself. In fact , the liver is capable of natural regeneration of lost tissue : as little as 25% of remaining liver can regenerate into whole liver again .

Liveris considered to be one of the most vital organs that functions as a centre of metabolism of nutrients such as carbohydrates, proteins, lipids and excretion of waste metabolites. Additionally, it is also handling the metabolism and excretion of drugs and other xenobioticsfrom the body thereby providing protection against foreign substances by detoxifying and eliminating them. The bile secreted by the liver has, among other things, plays an important role in digestion. Hepatic disease (Liver disease) is a term that affects the cells, tissues, structures, or functions of the liver. Liver has a wide range of functions,

including detoxification, protein synthesis, andproduction of biochemical necessary for digestion and synthesis as well as breakdown of small and complex molecules, many of which are necessary for normal vital functions. The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. Therefore, maintenance of a healthy liver is essential for the overall well-being of an individual. Liver cell injury caused by various toxicants such as certain chemotherapeutic agents, carbon tetrachloride, thioacetamide etc., chronic alcohol consumption and microbes is well-studied. [51]

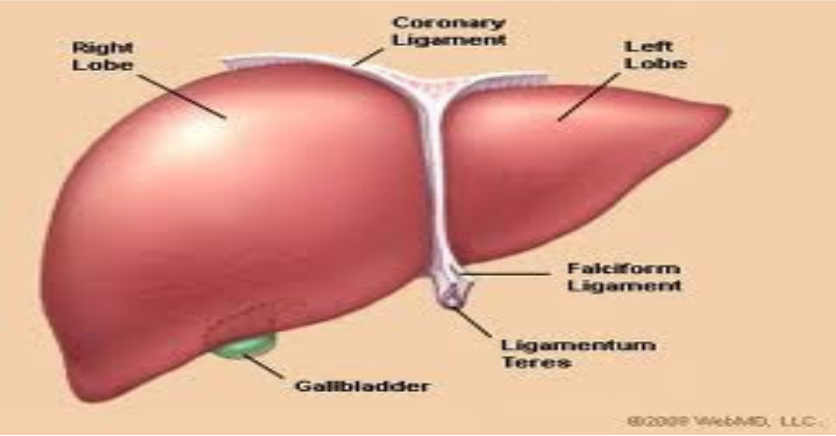


Fig.no.6

HISTOPATHOLOGICAL ALTERATION IN HEPATIC DAMAGE

The liver reacts with eight different types of responses to injury towards variety of metabolic, toxic, microbial circulation and neoplastic insults. Damage from toxic or immunologic insult may cause hepatocytes to take on a swollen and edematous appearance (ballooning degeneration) with irregularly clumped cytoplasm and large clear spaces. Alternatively, retained biliary material may impart a diffuse foamy swollen appearance to the hepatocyte (foamy degeneration). Substances may accumulate in viable hepatocytes including iron and copper. Accumulation of fat droplets within hepatocytes is known as ‘steatosis’ and appear in such conditions as alcoholic liver disease and acute fatty liver of pregnancy. A single large droplet that displaces the nucleus (macrovesicularsteatosis) may be seen in the alcoholic liver or in the liver of obese or diabetic individuals.

Necrosis is defined as focal death along with degradation of tissue by hydrolytic enzymes liberated by cells. Various agents such as hypoxia, chemical, physical agents, microbial agents and immunological injury can cause necrosis. Two essential changes bring about irreversible cell injury in necrosis is cell digestion by lytic enzymes and denaturation of proteins. These processes are morphologically identified by characteristic cytoplasmic and nuclear changes in necrotic cell. The cytoplasm appears homogeneous and intensely eosinophilic. Occasionally, it may show vacuolation or dystrophic calcification. The nuclear changes include condensation of nuclear chromatin which may either undergo dissolution or fragmentation into many granular clumps. Centrilobular necrosis frequently exhibits a zonal distribution. The most obvious necrosis of hepatocytes immediately around the terminal hepatic vein, an injury that is characteristic of ischemic injury occurred for a number of drug and toxic reactions. A variable mixture of hepatocellular death and inflammation is encountered. The hepatocyte necrosis may be limited to scattered cells within hepatic lobules. Bridging necrosis is more severe inflammatory injury; necrosis of continuous hepatocytes may span adjacent lobules in a portal to portal, portal to central and central to central fashion. Sub massive necrosis of entire lobules or most of the liver is usually accompanied by hepatic failure. With disseminated candidal or bacterial infection, macroscopic abscesses may occur. In fat necrosis, the necrosed fat cells have cloudy appearance, surrounded by an inflammatory reaction. Formation of calcium soaps is identified in the tissue sections as amorphous, granular and basophilic material. Microscopically, fibrinoid necrosis is identified by brightly eosinophilic, hyaline-like deposition in the vessel wall or on the hepatocytes[52]

LIVER FUNCTION TESTS

1. SERUM ENZYME ASSAYS

Determination of certain serum enzymes is considered useful in various types of liver injury , whether hepatocellular or cholestatic, as well as in quantitifying liver damage[53,54].

Serum glutamate oxaloacetate transaminase (SGOT)

This enzyme is released from damaged liver, heart, muscle, kidney or brain cells. Its normal serum level is up to 46 IU/L at 37°C. T levels are 10 to 200 fold elevated in patients with acute hepatic necrosis, viral hepatitis, CCl_4 and drug induced poisoning.

SGOT levels are also elevated by 10 fold in patients of post hepatic jaundice, intra hepatic cholestasis and less than 10 fold in alcoholic and hepatic steatosis.

Serum glutamate pyruvate transaminase (SGPT) test

This enzyme is released from damaged liver cells. Normal serum level of SGPT is up to 49 IU/L at 37°C and its levels are very high in patients of viral hepatitis and hepatic necrosis, 10 to 200 fold higher in patients of post hepatic jaundice, intra hepatic cholestasis and below 10 fold in patients of metastatic carcinoma, cirrhosis and alcoholic hepatitis.

Serum alkaline phosphates test

Elevated levels of alkaline phosphates, an enzyme found in the bile, usually indicate an obstruction of bile flow, liver injury, or certain cancers. Elevation in normal serum alkaline phosphatase (range 3-13 King Armstrong units/dl or 25-85 IU/dl) activity is found in diseases of bone, liver and in pregnancy. In the absence of bone disease or pregnancy, an elevated serum alkaline phosphatase level generally reflects hepatobiliary disease. The greatest elevation (3-10 times of normal) occurs in biliary tract obstruction. Slight to moderate increase is seen in parenchymal liver diseases such as hepatitis, cirrhosis and metastatic liver disease.

Serum total protein and albumin test

Routinely estimated total proteins are in the normal range of 5.5 to 8 g/dl. The blood levels of plasma protein are decreased in extensive liver damage. Albumin (normal range 3.5 to 5.0 g/dl) synthesized in the liver constitutes a major part of the total proteins in the body and the other part being globulin. A low serum albumin concentration suggests chronic liver disease. Albuminaemia may occur in liver diseases caused by significant destruction of hepatocytes. Hyperglobulinaemia may be present in chronic inflammatory disorders such as in cirrhosis and in chronic hepatitis.

Serum total and direct bilirubin test

Each day about 7.5 g of hemoglobin is catabolized with the corresponding production of 250 mg of bilirubin. Normally 0.25 mg/dl of conjugated bilirubin is present in the blood of an adult. Normal range for total bilirubin is from 0.2 to 1.2 mg/dl. Bilirubin level rises in diseases of hepatocytes, obstruction to biliary excretion into dueodenum, hemolysis and in defects of hepatic uptake and conjugation of bilirubin such as in Gilberts disease. Elevated levels of bilirubin often indicate an obstruction of bile flow or a defect in

the processing of bile by the liver. If the direct or conjugated bilirubin is low, while the total bilirubin is high, this reflects liver cell damage or bile duct damage.

Serum bile acids

Fasting bile acids concentrations in excess of 15μmol/L can be the result of hepatobiliary disease. The probability of hepatobiliary disease is high if fasting bile acid concentration is greater than 25-30 μmol/L.

Serum lipid profile test

Liver toxicants cause disturbances in synthesis and metabolism of triglycerides, cholesterol and lipoproteins, thus damaging the basic resource for living cells. Cholesterol and bile salts are synthesized by liver cells thus liver intoxication decreases level. Normal range of cholesterol levels are up to 200 mg⁄dl or lower for a total count, but it is important to check HDL and LDL levels for a better analysis. Fatty degeneration of the liver causes increased triglyceride (normal range >150 mg/dl) content in the blood.

γ- glutamyltransferase test

Gamma glutamyltransferase (γ -GT) also known as γ- glutamyltranspeptidase is a microsomal enzyme with wide tissue distribution. This enzyme is produced by the liver, pancreas, kidneys and released into the blood when these organs are injured. The normal γ-GT serum level is up to 26 U/L. In alcoholics with liver abscess, it is increased by 2-5times.

5-Nucleotidase test

Normal range of 5-Nucleotidase is 2 to 17 U/L. The liver releases this enzyme when injured due to bile duct obstruction or impaired bile flow. Greater than normal values indicate liver cell destruction, liver ischemia, necrosis, hepatitis, cholestasis or liver tumor.

Lactic dehydrogenase test

Reference ranges for total LDH vary from laboratory to laboratory. Normal values are also higher in childhood. For adults, in most laboratories, the range can be up to approximately 200 U/L, but is usually found within 45-90 U/L. When disease or injury affects tissues containing LDH, the cells release it into the bloodstream, identified as higher than normal levels. The LDH is also elevated in heart attack, diseases of the liver, in certain types of anemia, and in cases of excessive destruction of cells, as in fractures, trauma, muscle damage and shock

Alpha-fetoprotein test

In adults, high blood levels (over 500 ng/ml) of AFP are seen in only three situations like hepatocellular carcinoma, germ cell tumors and metastatic cancer in the liver. Also, pregnant women carrying babies with neural tube defects may have high levels of AFP.

Mitochondrial antibodies test

AMA are present in less than 1 % of normal people and in less than 5% of patients with systemic lupus erythematosusrheumatoid arthritis and other autoimmune diseases. Patients with extra hepatic biliary obstruction, Wilson's disease, hemochromatosis, and alcoholic cirrhosis rarely have elevated titers. The presence of mitochondrial antibodies remains a useful diagnostic tool in the differential diagnosis between primary biliary cirrhosis and extra hepatic biliary obstruction.

Prothrombin time test (PTT)

This test measures the time it takes for blood to clot. Blood clotting requires vitamin K and coagulation factors like II, V, VII, and IX synthesized in the liver. Liver cell damage and bile flow obstruction can both interfere with proper blood clotting.[54]

ROLE OF HERBAL THERAPY IN HEPATOPROTECTIVE ACTIVITY

Liver diseases have become one of the major causes of morbidity and mortality in man and animals all over globe and hepatotoxicity due to drugs appears to be the most common contributing factor.Liver cell injury caused by various toxicants such as certain chemotherapeutic agents, carbon tetrachlo-ride, thioacetamide etc., chronic alcohol consumption and microbes. Among the many diseases that can affect the liver the most common is 'viral hepatitis' (Inflammation of liver caused by viral infection)[55]. Hepatitis can be caused by drugs, viruses, bacteria, mushrooms, parasites like amoebas or giardiasis. The Indian Tradi-tional Medicine like Ayurveda, Siddha and Unani are predominantly based on the use of plant materials. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness.[56]. The association of medical plants with other plants in their habitat also influences their medi-cinal values in some cases. One of the important and well-documented uses of plant-products is their use as hepatoprotective agents. Hence, there is an ever in-creasing need for safe hepatoprotective agent[57].

Hepatoprotective herbs

A large number of plants and formulations have been claimed to have hepatoprotective activity. Nearly 160 phytoconstituents from 101 plants belonging to 55 families have been claimed to possess liver protecting activity. In India, more than 87 plants are used in 33 patented and proprietary mul-ti-ingredient plant formulations[(58)]. Liver protective plants contain a variety of chemical constituents like phenols, Coumarins, Lignans, essential oil, monoterpenes, carotinoids, glycosides, flavonoids, organic acids, lipids, alkaloids and xanthenes. Therefore a large number of plants and formulations have been claimed to have hepatoprotectiveactivity.Inspite of the tremendous advances made, no significant and safe hepatoprotective agents is available in modern therapeutics, so the development of plant based hepatoprotective drugs has been given importance in the global market[(59)].

ETHNO- MEDICINAL PLANTS USED IN LIVER DISEASES[60]

Table no.1

SL NO	PLANT NAME	FAMILY	PARTS OF THE PLANT USED
1	*Orthosiphonstamineus*	Lamiaceae	Leaves
2	*Baliospermummontanum*	Euphorbiaceae	Roots
3	*Tridax procumben*8	Asteraceae	Leaves
4	*Glycyrrhizaglabra*Linn.	Fabaceae	Root powder
5	*Phyllanthusniruri*	Euphorbiaceae	Leaves and fruits
6	*CochlospermumPlanchoni*	Coclospermaceae	Rhizomes
7	*Saururuschinensis*	Saururaceae	Whole plant
8	*FructusSchisandraechinensis (LFS)* with *Astragalus polysaccharides(APS)*	Magnoliaceae	Dried fructus
9	*Cordiamacleodii*	Boraginaceae	Leaves
10	*Arachniodesexilis*	Dryopteridaceae	Rhizomes
11	*Momordicadioica*	Cucurbitaceae	Leaves
12	Swertiamarin isolated from *EnicostemmaAxillare*	Gentianaceae	Whole plant
13	*Asparagus racemosus*	Liliaceae	Whole plant
14	*Tephrosiapurpurea L.*	Fabaceae	Aerial parts
15	*Tecomella undulate*	Bignoniaceae	stem Bark
16	*Cassia fistula*	Leguminosae	Leaf
17	*Gentianaolivieri*	Gentianaceae	Aerial parts

18	*Amaranthusspinosus*	Amaranthaceae	Whole plant
19	*Apiumgraveolens*and	Apiaceae	Seeds
20	*Boerhaaviadiffusa*	Nyctaginaceae	Roots
21	*HygrophilaAuriculata*	Acanthaceae	Seeds
22	*Clerodendruminerme*	Verbenaceae	Leaves
23	*Zanthoxylumarmatum*	Rutaceae	Bark
24	*Gundeliatourenfortii*	Asteraceae	Fresh edible stalk
25	*Cassia occidentalis*	Caesalpiniaceae	Leaves
26	*Kalanchoepinnata*Pers	Crassulaceae	Leaves
27	*Luffaechinata*	Cucurbitaceae	Fruits
28	*PhyllanthusamarusSchum. et.* Thonn.	Euphorbiaceae	Aerial part
29	*Schouwiathebica*	Arecaceae	Aerial parts
3O	*Thunbergialaurifolia*Linn.	Acanthaceae	Leaves

1) ***Yunchi Endo.,et al.*** investigated a new tetracyclic cyclopropane derivative favelanone having cytotoxic activity from Cnidoscolus phyllacanthus and the structure was elucidated on the basis of its spectroscopic data.(60)
2) ***J.c.o santos., et al.*** investigated the physicochemical and thermal analysis of favelone and its by-products extracted from Cnidoscolus phyllacanthus. This work presents the result of physicochemical and thermal characterization of the favelone seed and its by-products (Cnidoscolus phyllacanthus).(61)
3) ***J.o.kuti.,et al.*** investigated the proximate composition and mineral content of two edible species of Cnidoscolus . Results showed the leafy parts of the species consists of large amounts of crude protein, crude fiber, Ca, K,Fe, ascorbic acid, and β-carotene. However there is significant difference in nutritional composition of fatty acid, protein and amino acid, the edible Cnidoscolus species leaves may be good dietary source of minerals and vitamins.(62)
4) ***Tadeu jose da silva peixoto sobrinho.,et al,*** evaluated the antiproliferative activity of Cnidoscolus quercifolius against HT-29,HEP-2 and NCI-H292 cells and reported that Cnidoscolus quercifolius shows inhibition percentage of cell growth and it is active against HT-29 and HEP-2.(63)
5) **MuthuGounderPalanivel,et al.,(2008)** has studied Ethanol extract of Pisoniaaculeata(EPA) was evaluated for hepatoprotective and antioxidant activities in rats. The plant extract (250 and 500 mg/kg, p.o.) showed a remarkable hepatoprotective and antioxidant activity against carbon tetrachloride (CCl_4)-induced hepatotoxicity as judged from the serum marker enzymes and antioxidant levels in liver tissues. CCl_4-induced a significant rise in aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin, gamma glutamate transpeptidase (GGTP), lipid peroxidase (LPO) with a reduction of total protein, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione S-transferase (GST). Treatment of rats with different doses of plant extract (250 and 500 mg/kg) significantly ($P<0.001$) altered serum marker enzymes and antioxidant levels to near normal against CCl_4-treated rats. The activity of the extract at a dose of 500 mg/kg was comparable to the standard drug, silymarin (50 mg/kg, p.o.). Histopathological changes of liver sample

were compared with respective control. Results indicate the hepatoprotective and antioxidant properties of *P. aculeata* against CCl_4-induced hepatotoxicity in rats.[64]

6) **Manokaran, et al.,(2008)** conducted to evaluate the hepatoprotective activity of hydroalcoholic extract of *Aervalanata* against paracetamol induced liver damage in rats. The hydro alcoholic extract of *Aervalanata*(600mg/kg) was administered orally to the animals with hepatotoxicity induced by paracetamol (3gm/kg). Silymarin (25mg/kg) was given as reference standard. All the test drugs were administered orally by suspending in 0.5% Carboxy methyl cellulose solution. The plant extract was effective in protecting the liver against the injury induced by paracetamol in rats. This was evident from significant reduction in serum enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin . It was concluded from the result that the hydro alcoholic extract of *Aervalanataa* possesses hepatoprotective activity against paracetamol induced hepatotoxicity in rats.[65]

7) **Vinodhini Singanan,et al.,(2007)** evaluated the hepatoprotective effect of bael leaves in alcohol induced liver injury in albino rats. The experiments were performed with four groups of animals. The experimental animals were administered with 30% ethyl alcohol for a period of 40 days and the fine crude plant leaves powder was fed to animals for next 21 days. The observed values of TBARS (Thiobarbituric acid reactive substances) in healthy, alcohol intoxicated and herbal drug treated animals. The results were compared with the standard herbal drug silymarin (133.04 µg/g tissue). From the experimental results they concluded that, the Bael leaves have excellent hepatoprotective effect.[66]

8) **Deepak. k. Das, et al.,(2007)** has studied the hepatoprotective effect of chloroform and methanol extract (CEIF and MEIF) of whole plant of *I. frutescens* (Linn.) by paracetamol-induced liver damage in rats. The degree of protection was measured by using biochemical parameters such as serum glutamate oxalate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), bilirubin and total protein. Further, the effects of both extracts on lipid peroxidation (LPO), glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) were estimated .CEIF and MEIF at a dose level of 250mg/kg and 500mg/kg produce significant ($P<0.05$) hepatoprotection by decreasing the levels of serum enzymes, bilirubin, and lipid peroxidation , while they significantly increased the levels of Glutathione (GSH),

superoxide dismutase (SOD) and catalase (CAT) in a dose dependent manner. The effects of CEIF and MEIF were comparable to that of standard drug, Silymarin. From this study, it can be concluded that the chloroform and methanol extract of *I.frutescens* is not only an effective hepatoprotective agent, but also possesses significant ($p<0.05$) antioxidant activity.[67]

9) **B.K. Chandan,et al.,(2007)** evaluated the Hepatoprotective potential of *Aloe barbadensis* against carbon tetrachloride induced hepatotoxicity. The shade dried aerial parts of *Aloe barbadensis* were extracted with petroleum ether (AB-1), chloroform (AB-2) methanol (AB-3) distilled water (AB-4). All the extracts were evaluated, AB-1 and AB-2 were observed to be devoid of any hepatoprotective activity. Out of two active extracts (AB-3 and AB-4), the most active AB-4 was studied in detail. AB-4 showed significant hepatoprotective activity against CCl_4 induced hepatotoxicity as evident by restoration of serum transaminases, alkaline phosphatase, bilirubin and triglycerides. From this study they found out that the aqueous extract of *Aloe barbadensis* is significantly capable of restoring integrity of hepatocytes indicated by improvement in physiological parameters, excretory capacity of hepatocytes and also by stimulation of bile flow secretion, also it did not show any sign of toxicity up to oral dose of 2 g/kg in mice.[68]

10) **Shanumugasundaram P, et al.,(2006)** has evaluated hepato protective and antioxidant effects of *hydrophilaauriculata* root against CCl_4 induced liver toxicity in rats and ferric thiocyanate (FTC) and thiobarbituric acid (TBA) method respectively . The activity was accessed by monitoring the various liver function tests , viz alanine amino transaminase , aspartate amino transaminase (AST) , alkaline phosphatase (ALP) , Total protein and total bilirubin. Furthermore hepatic tissues were subjected to histopathological studies. They concluded that there was a significant hepatoprotective activity and antioxidant activity for aqueous extract of the roots of *hygrophilia auriculata*.[69]

11) **Sharmila Upadhya, et al.,(2004)** done a study of hypoglycemic and antioxidant activity of *Aegle marmelos* in alloxan induced diabetic rats. Animals were divided into three groups. Group I : Control; Group II : Diabetic rats; and Group III : Diabetic rats administered AML. Glucose, urea and glutathione-S-transferase (GST) in plasma,

glutathione (GSH) and malondialdehyde (MDA) levels in erythrocytes were estimated in all the groups at the end of four weeks. There was a decrease in blood glucose at the end of four weeks in group III animals compared with group II, however it did not reach the control levels. There was an increase in erythrocyte GSH and a decrease in MDA in group III as compared to group II. The plasma GST levels were raised in diabetic rats when compared to controls. In the group III animals, there was a decrease in GST as compared to group II.[70]

12) **Al-Qarawi, et al.,(2001)** has evaluated the aqueous extract of the *Adansoniadigitata*(Linn.) pulp was tested for hepatoprotective activity against chemical toxicity with CCL_4 in rats. The aqueous extract exhibited significant hepatoprotective activity. [71]

13) **Rawat ,et al ., (1997)** studided hepatoprotective activity of *Boerhaavia diffusa* Linn.roots a popular Indian ethanomedicine . In this study the effects of seasons, thickness of roots and form of dose were studied for their hepatoprotective action to prove the claims made by the different diameters tribes of india. The hepatoprotective activity of roots of different diameters collected in three seasons , rainy, summer, and winter , was examined in thioacetamide intoxicated rats . The result showed that an aqueous extract (2ml/kg) of roots of diameter 1-3 cm, collected in this month may (summer) exhibited marked protection of a majority of serum parameters , viz. SGOT, SGPT.[72]

14) **Anubhasingh ,et al.,(1995)** has evaluated the hepatoprotective activity of *Apiumgaveolens* and *Hygrophila auriculata* against paracetamol and thioacetamide intoxication in rats . In this study the rat liver was damaged by a single dose of paracetamol (3kg/kg:p.o) or thioacetamide (100mg/kg; s.c). They were monitoring several liver function tests, Viz. serum transaminase (SGOT and SGPT) , alkaline phosphatase, sorbitol dehydrogenase , glutamate dehydrogenase, and bilirubin in serum . They concluded that there was a significant hepatoprotective activity of the methanolic extract of the seeds of *ApiumGraveolens* and *Hygrophila auriculata*.[73]

Herbal drugshave become increasingly popularand their use is widespread. Licensing Regulations and pharmacovigilance regarding herbal products are still incomplete and clearcut proof of their efficacy in liver diseases is sparse. Nevertheless , a number herbals show promising activity including silymarin for antifibrotic treatment , *phyllantusamarus*in chronic hepatitis B, Glycyrrhizin to treat chronic viral hepatitis ,and a number of herbal combinations fromchina and Japan that deserve testing in appropriate studies. The focus of the present work is to elucidate Ayurvedic concept of liver cirrhosis, using herbal remedies.

In traditional medicine , natural, crude phytoextracts considered as alternative medicines, because some natural constituents present in them counter balance.The side-effects ofsynthetic medicines. It is therefore obvious that the therapeutic potential and risk efficacy or traditional medicinal plants is based on the direct assessment of Phytoextracts as well as effects of their purified compounds. Plants have played a significant role in maintaining human health and improving the Quality of human life for thousands of years and have served human wellasvaluable components of medicine , seasoning, beverages , cosmetics and dyes. Herbal medicines arebased on the premise that plant contains natural substances that can promote health and alleviateillness. In recent times ,focus on plants research has increased all over the world and a large body ofevidence has collected to show immense potential of medicinal plants used in various traditional systems. Today ,we are witnessing a great deal of public interest in the use of herbal remedies . Furthermore many Western drugs hadtheir origin inplant extracts. There are many herbs ,which are predominantly Used to treat cardiovascular problems, cancer, central nervous system, digestive and metabolic disorders. Give their potential to produce significant therapeutic effect,they can be useful as drug or supplement in the treatment , management of various diseases. Herbal drugs or medicinal plants , their extracts and isolated compounds have demonstrated spectrum of biological activitiessuch have been used and continued tobe used as medicine in folk fore or food supplement for various disorders. Ethanopharmacological studies on such herb,medicinally importantplants continue to interest investigators throughout theworld.

Herbal drugs are traditionally used in various parts of the world to cure various diseases alternative systems of medicine like Ayurveda, Siddha and Unani are very famous Medical practices in traditional medicines . It providesa holistic approach to treat Hepaticdisorder with success. Accordingto world health organization (WHO), manypeopledie every year due to hepatic disorders.Liverdiseases are large public health problem in the world. Towards these pathologies Which may appear with multiple diversified cause and appearances , a modern medicine does not find any curative treatments . A large proportion of the Indian population for their physical and psychological health needs depend on traditional system of medicine. Medicinal plants have become the focus of intense study in term of conservation as to whether their traditional uses are supported by actual pharmacological effects or merely based on folkfore .

The Indian is the birthplace of renownedsystem of indigenousMedicines suchAs Ayurveda siddha and unani. The country is enriched with flora there for plant remedies can be used since ancient times for the treatment of human ailment . There is no rational therapy available for treating liver disorder and still challenge to the modern medicine. The modern medicines have little to offer for alleviation of hepatic ailments whereas most important representative is of phytoconstituents.

Many Indian ethno botanic traditions propose a rich repertory of Medicinal plants used by the population for the treatment of liver disease (jaundice,liver Gallstone, hepatitis etc.)However, there weren't enough scientific investigations in theHepatoprotectiveactivities conferred to these plants. Recent progress in the study of such plants has Resulted in the isolation about 170 different phytoconstituents from plants belonging to about 55 families which exhibit hepatoprotective activity.

Hepatotoxicity can affect hundreds of millions of peopleworldwide. It is the common non- neo plastic cause of death among hepatobiliary and digestive disorders serious side effects, the cost of the modern medicine and improper channel of treatment and competitive efficacy of natural products made the person through the world to look for classical plant drugs for the treatment of hepatotoxicity .

In view of the pharmacological and biological properties and chemical constituents of the plantC*nidoscolusphylanthus,* it was decided to an attempt is made to find out the extent of hepatoprotective and antioxidant activity of plant.

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AIM OF WORK:

The present study was undertaken to determine theHepatoprotectiveand antioxidant activity and also the evaluation of the various biochemical parameters of *CnidoscolusPhyllacanthus*.

PLAN OF WORK

The Hepatoprotectiveand antioxidant activity of *CnidoscolusPhyllacanthus* animal model is not yet evaluated till date. Therefore this study has proposed to perform with the following objectives.

OBJECTIVE

- To evaluate the Hepatoprotective and antioxidant activity of ***CnidoscolusPhyllacanthus***Linnin animal models
- To compare the antioxidanteffects observed with the marketed standard product Vitamin E.

HEPATOPROTECTIVE AND ANTIOXIDANT WORK IS PLANNED TO CARRY OUT AS OUTLINEDBELOW

- Extraction of whole plant of ***CnidoscolusPhyllacanthus***using Soxhlet apparatus using hydro alcohol as solvent.
- In-vivo evaluation of the plant extract of ***CnidoscolusPhyllacanthus***
- Selection, grouping and acclimatization of the animals.

I. Induction of hepatotoxicity by D-galactosamine.
II. Treatment protocol with the extract.
III. Study of serum parameters analysis.
IV. Bio-chemical analysis viz- serum analysis and histopathological studies.
V. Statistical analysis.

PLANT PROFILE

Botanical Name :cnidoscolus quercifolias

Synonym : cnidoscolus phyllacanthus

Family : Euphorbiaceae

BOTANICAL CLASSIFICATION

Kingdom :Plantae

Class :Eudicots

Subclass : Rosidsae

Order :Malpighiales

Family :Euphorbiaceae

Subfamily :Crotonoideae

Tribe: Manihoteae

Genus:cnidoscolus

Species:c.Quercifolias

CHEMICAL CONSTITUENTS[74,75]

- Protein, crude fibre, fat, carbohydrate
- Mineral constituents like Ca, Mg, Na, Mn, Fe,Cu, Zn are present.
- Terpenoids like phyllacanthone, 3β-O-cinnamoyl-lupeol are present
- The active tetracyclic compounds isolated from the plant are Faveline, Faveline methyl ether, Deoxofaveline,Isofavelol, Favelanone, Neofavelanone.[60]
- Cyanogenic contents i.e; HCN are also seen in this plant.
- It also contains coumarins, flavonoids and tannins.

HABITAT AND DISTRIBUTION

It is indigenous to the semiarid region of northeastern Brazil. It is seen in dry regions of tropics.

DESCRIPTION

It is large, leafy, throny shrub. It is aarboreal plant that has short trunk, cylindrical and branched from the base.

USES[63]

The extracts of cnidoscolus have high inhibition percentage of cell growth. These species are commonly used to treat tumors and inflammation. The roots, bark and latex are used for the treatment of inflammatory processes, genitourinary and in general as antiseptic, dermatologic and ophthalmic. It is also used to treat kidney diseases, urinary infections, contusions, fractures, wounds, warts and hemorrhoids. It is also a good sourse of protiens, vitamins and minerals.

CnidoscolusPhyllacanthus

Fig.no. 7

SOLVENT EXTRACTION (HOT PERCOLATION METHOD)

Preparation of petroleum ether, chloroform and ethanolic extracts of cnidosolusphyllacanthus.

EQUIPMENT USED

Soxhlet apparatus

MATERIALS USED

Petroleum ether

Chloroform

Ethanol

Shade dried Cnidoscolusphyllacanthus

METHOD

The Cnidoscolusphyllacanthus plant was collected and identified. The leaf was cut down into small pieces, shade dried and powdered to get moderately coarse powder, which is sieved under mesh. About 500gm of dry powder was extracted with petroleum ether, chloroform and ethanol at 60-70^{o}c by hot continuous percolation using soxhlet apparatus. The extraction was continued for 72hrs. the petroleum ether, chloroform and ethanolic extract was filtered and concentrated to a dry mass by using vaccum distillation the petroleum ether extract(4gms) was obtained as dark green residue. The chloroform extract (5gms) was obtained as dark brown residue. The ethanolic extract (7.2gms) was obtained as dark brown residue.

EXPERIMENTAL MODELS

For the study of hepatoprotective and antioxidant activity an animal model was added that would satisfy the following conditions.

- The animal should develop liver toxicity rapidly and reproducibly
- Pathological changes in the site of induction should result from liver damage.
- The symptoms should be ameliorated or prevented by a drug treatment effective in human beings .
- The drug tested should be administered orally
- Drug dosage approximate the optimum therapeutic range for human, scaled the test animal weight.

LABORATORY ANIMAL MODELS

INDUCTION OF HEPATO TOXICITY AND FREE RADICALS IN ANIMAL MODEL

EXPERIMENTAL PHARMACOLOGICAL STUDIES IN ANIMAL LIVER

To investigate and evaluate hepatoprotective substance, it is customary to subject animals to a range of toxic agents. These hepatotoxicants include carbon tetrachloride, D-galactosamine, thioacetamide, ethanol, aflatoxin B1, alpha amanitine, phalloidin, cadmium, paracetamol, hydrazine, halothane, isoniazid etc that causes damage of rat liver, resulting in biochemical and histopathological changes. Different toxicants used for experimental liver damage with dose range, route, vehicle and detailed schedule of treatment.

Induced by ethanol

The basic mechanism in the induction of hepatotoxic by ethanol is principally metabolized to acetaldehyde in the liver and seldom in other tissue by alcohol dehydrogenase as well as CAT(catalase). Acetaldehyde is further oxidized into acetate by acetaldehyde dehydrogenase oxidase.,leading to the generation of ROS/free radical. Ethanol is also oxidised by a microsomal Ethanol oxidising system($CYP2E_1$) to acetaldehyde and 1- hydroxyethyl radical especially following chronic ethanol consumption by which $CYP2E_1$ is induced. Excessive alcohol intake results indisequilibriumin iron homeostasis

and iron overload which further enhance oxidative stress by catalyzing the formation of more noxious hydroxyl free radical**.** Hence induction of $CYP2E_1$and iron overload by ethanol are critical path way by which ethanol generates a state of oxidative stress in hepatocytes[76].

Induced by paracetamol

The mechanism by which over dosage with paracetamol leads to hapatocellular injury and death involves its conversion to the toxic NAPQ1(N- acetyl – Para benzoquinone imines) metabolite. The glucoronide sulfa conjugation pathways become saturated and increasing amount undergo CYP-mediated N - hydroxylation to form NAPQI. This is eliminated rapidly by conjugation with GSH and then further metabolized to a mercapturic acid and excreted into urine. In the setting of paracetamol overdose, hepatocellular level of GSH become depleted . The highly reactive NAPQ1 metabolite binds covalently to cell macromolecules leading to dysfunction of enzymatic system and structural and metabolic disarray further more depletion of intracellular GSH renders the hepatocytes highly susceptible to oxidative stress and apoptosis.[77]

Induced by CCl_4

CCl_4 induce liver damage by producing free radical intermediates. CCL_4 is converted to trichloromethyl radical (CCl_3) by the P-450 system. Which in turn is converted to Peroxy radical (CCl_3O_2) which causes the damage.

Induced by D- galactosamine

Galactosamine is a hexosamine derived from galactose. It causes liver injury via the generation of free radicals and depletion of UTP nucleotides. Galactosamine produces the hepatotoxic effect by selectively reducing the uridine pool in hepatocytes. This intern inhibits mRNA and protein synthesis, alters the composition of cellular membranes and finally leads to cellular damage as a result of lipid per oxidation. The hepatocyte death is represented as apoptosis and subsequently necrosis. Other mechanism of galactosamine hepatotoxicity stated that galactosamine increases intestinal permeability and subsequently facilitates bacterial translocation to the liver. Lipo polysaccharides activate kupffer cells to secrete tumor necrosis factor-α ,which raises expression of intercellular adhesion molecule 1 in endothelial cells and this promotes the adhesion of polymorphonuclear cells to vascular and

hepatic endothelial cells , leading to polymorphonuclear infiltration and hepatocyte damage. Galactosamine induces rise in SGOT, SGPT and total bilirubin where as decrease in total protein. Galactosamine shows pathological changes like moderate degeneration and necrosis of hepatocyte**.**

Induced by INH+RIF

During metabolism of INH, Hydrazine can be produced by both directly (From INH) and indirectly (from acetyl hydrazine) . The direct pathway involves hydrolysis of the amide bond of INH to produce Iso nicotinic acid and hydrazine. The indirect pathway involves acetylation of INH to acetyl-INH by N-acetyl transferase hydrolysis of acetyl INH to Isonicotinic acid and acetyl hydrazine , and hydrolysis deacetylation to hydrazine. Hydrazine is a known hepatotoxin.

MATERIALS AND METHODS

ANIMALS : Albino wistar rats (180-220gm)

CHEMICALS : D- galactosamine

: Vitamin E

: Ethanolic extract of Cnidoscolusphyllanthus

SELECTION AND ACCLIMITIZATION OF ANIMALS

Albino rats of wistar strains weighing between 180-220gm were produced from animal experimental laboratory, and used throughout the study. They were housed in micronylon boxes in a control environment(temp 25+-2^0c) and 12 hrs dark\ light cycle with standard laboratory diet and water ad libitum . The study was conducted after obtaining Institutional Animal Ethical Committee clearance. As per the standard practice, the rats were segregated based on their gender and quarantined for 15 days before the commencement of the experiment. They were fed on healthy diet and maintained in hygiene environment in our animal house.

METHADOLOGY

Treatment protocol

The acclimatized animals were divided into 5 groups of each 6 animals, designated as

- Group 1: Served as normal control and receive normal diet and water.
- Group 2: Toxic control received 25mg/kg of D-galactosamine through I.P for 21 days.[78]
- Group 3: Standard control received 25mg/kg of vitamin E orally for 21 days.
- Group 4: The treatment control received 200mg/kg of Ethanolic extract of *Cnidoscolusphyllanthus*orally for 21 days.
- Group 5 :The treatment control received 400mg/kg of Ethanolic extract of *Cnidoscolusphyllanthus* orally for 21 days.

PREPERATION OF DRUGS

- Ethanolic extract of *Cnidoscolusphyllanthus*was dissolved in 20ml of sterile water and was administered orally at a dose of 200mg/kg and 400mg/kg/rat.
- D-Galactosamine was diluted in sterile water and administered I.P at a dose of 25mg/kg/rat.
- Vitamin E was diluted in sterile water and administered orally at a dose of 25mg/kg

METHODOLOGY

On day 22 after 24 hrs of Galactosamine administration animals in all the groups were humanely sacrificed using Ketamine HCl and 4ml of blood was withdrawn by cardiac puncture and allowed to clot for 30mins at room temperature. The serum was separated by using cooling centrifuge and used for the assay of marker enzymes viz AST, ALT, ALP, TP, TB,GGPT and total albumin. The livers were dissected out immediately, washed with ice-

cold saline and 10% homogenates in phosphate buffer solution (PH 7.4) were prepared Liver homogenate was used for the assay of Lipid peroxidation (Lpo) while some fraction of homogenates were centrifuged at 7000rpm for 10 min at 4^0 C using refrigerated centrifuge, and the supernatants were used for the assay of Superoxide dismutase (SOD), catalase(CAT), Glutathione peroxidase(GPx). Some portion of liver from each group was aseptically excused and stored in 10% formalin for histopathologicalstudies[79].

STATISTICAL ANALYSIS

The Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by NewmannKeul's multiple range tests. The values are represented asMean $\pm$ SEM. Probability value at $P < 0.01$ was considered as statistically significant.

Table. No:2

Effect of *Cnidoscolusphyllanthus*and *VitaminE* pre-treatment on biochemical parameters of the rats intoxicated with D-Galactosamine.

Group. No.	TREATMENT DOSE (mg/Kg)	AST (IU/mL)	ALT (IU/mL)	ALP (IU/mL)	TP (gm/dl)	TB (mg/dl)	GGTP (mg/dl)	Total Albumin(g/dl)
I	Normal control 10ml/kg normal saline	44.40± 1.52	30.09± 1.49	23.68± 1.30	5.15± 0.08	1.92± 0.08	96.90± 2.75	3.80± 0.16
II	Toxic control 25mg/kg D-galactosamine	*a 105.90 ± 2.40	*a 94.49± 1.05	*a 144.10± 2.35	*a 3.16± 0.22	*a 4.40± 0.26	*a 173.42± 2.90	*a 2.20± 0.07
III	Standard control Vitamin E 25mg/kg	*b 60.10± 1.20	*b 40.56± 1.06	*b 56.4± 1.70	*b 3.90± 0.08	*b 2.8± 0.15	*b 122.20± 1.95	*b 2.90± 0.05
IV	Treatment control EECP 200mg/kg	*b 68.65± 1.46	*b 54.82± 2.72	*b 65.86± 2.30	*b 4.60± 0.25	*b 3.30± 0.20	*b 136.30± 3.04	*b 2.54± 0.04
V	Treatment control EECP 400mg/kg	*b 62.45± 1.15	*b 47.94± 0.97	*b 58.50± 1.95	*b 4.05± 0.26	*b 2.95± 0.18	*b 130.94± 1.23	*b 2.30± 0.09

- Valuesare expressed as Mean ± SEM.
- Values are found out by using one way ANOVA followed by Newmannkeul's multiple range tests.
- *a – values are significantly different from Normal control at P< 0.01.
- *b – values are significantly different from Toxic control(G2) at p< 0.01.

Table.No:3

Effect of*Cnidoscolusphyllanthus*and Vitamin E pre-treatment on biochemical liver parameter in D-Galactosamine induced hepatotoxicity.

Group. No.	TREATMENT DOSE (mg/Kg)	SOD (U/mg) Protein	CATALASE (U/mg) Protein	GPX (U/mg) Protein	MOA (U/mg) Protein
I	Normal control 10ml/kg Normal saline	132.25± 2.40	290.40± 2.40	1.10± 0.05	3.90± 0.17
II	Toxic control 25mg/kg D-galactosamine	*a 68.20± 1.65	*a 190.75± 2.70	*a 0.40± 0.02	*a 7.40± 0.12
III	Standard control Vitamin E 25mg/kg	*b 118.05± 2.80	*b 260.45± 1.92	*b 0.85± 0.02	*b 4.50± 0.14
IV	Treatment control 200mg/kg EEPL	*b 96.50± 1.60	*b 230.05± 1.80	*b 0.55± 0.02	*b 5.60± 0.28
V	Treatment control 400mg/kg EEPL	*b 105.65± 2.62	*b 240.75± 2.65	*b 0.74± 0.02	*b 4.80± 0.08

- Values are expressed as Mean ± SEM.
- Values are finding out by using one way ANOVA followed by Newmannkeul's multiple range tests.
- *a – values are significantly different from Normal control at P< 0.01.
- *b – values are significantly different from Toxic control(G2) at p< 0.01.

TABLE NO.4

EFFECT OF EECP ON THE LEVELS OF NON ENZYMATIC ANTIOXIDANTS IN THE LIVER TISSUE OF D-GALACTOSAMINE –HEPATOTOXIC AND CONTROL RATS

GROUPS	GLUTATHIONE MG/100G TISSUE	VITAMIN-C MG/100G TISSUE	VITAMIN-E MG/100G TISSUE
Normal control 10ml/kg normal saline	132.60±3.45	0.82±0.08	5.92±0.60
Toxic control 25mg/kg D-galactosamine	73.55±1.70*a	0.30±0.02*a	2.40±0.30*a
Standard control Vitamin E 25mg/kg	110.32±2.70*b	0.74±0.07*b	5.60±0.55*b
Treatment control EECP 200mg/kg	98.05±2.16*b	0.60±0.04*b	4.92±0.50*b
Treatment control EECP 400mg/kg	91.90±1.95*b	0.69±0.06*b	5.02±0.48*b

- Values are expressed as Mean ± SEM.
- Values are found out by using one way ANOVA followed by Newmannkeul's multiple range tests.
- *a – values are significantly different from Normal control at P< 0.01.
- *b – values are significantly different from Toxic control (G2) at p< 0.01.

HISTOPATHOLOGICAL STUDIES OFLIVER TISSUE

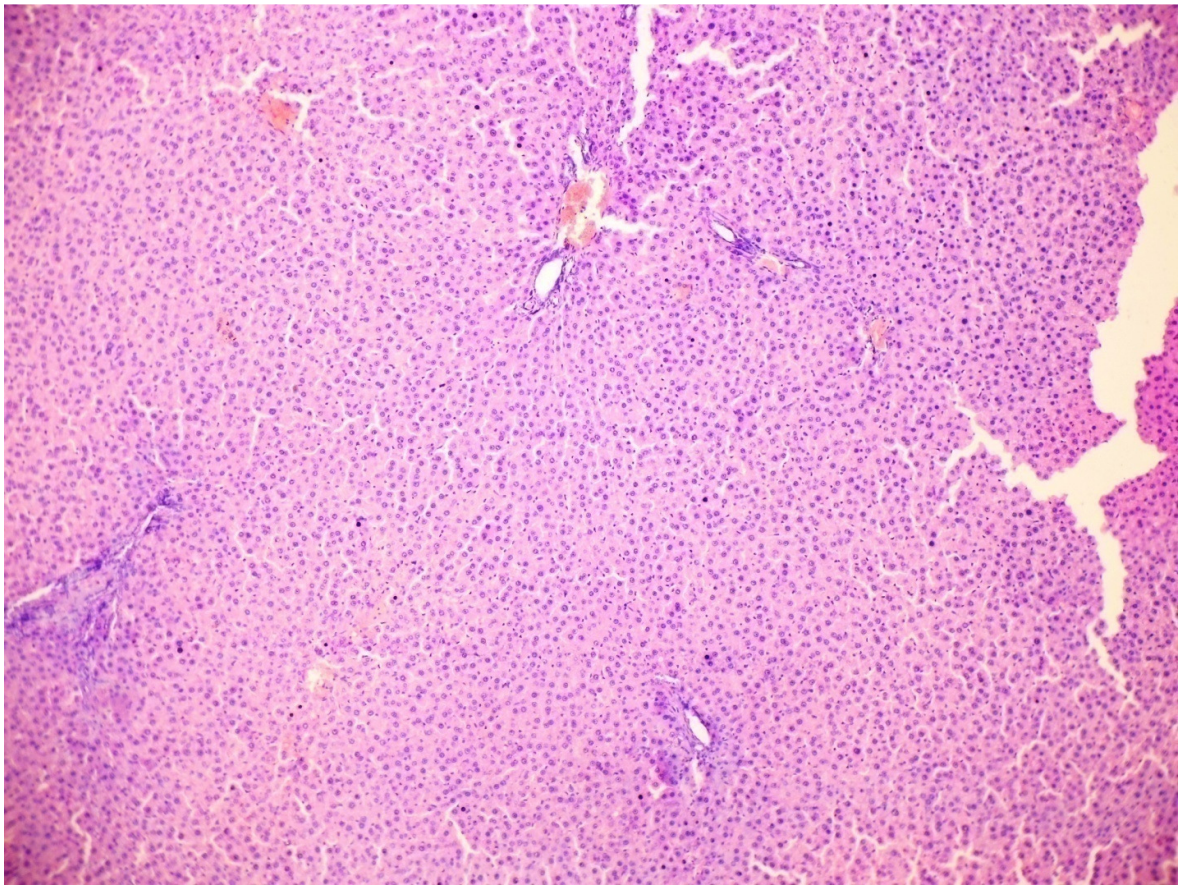

Fig.no.8

Liver section of GP_1 (Normal control)

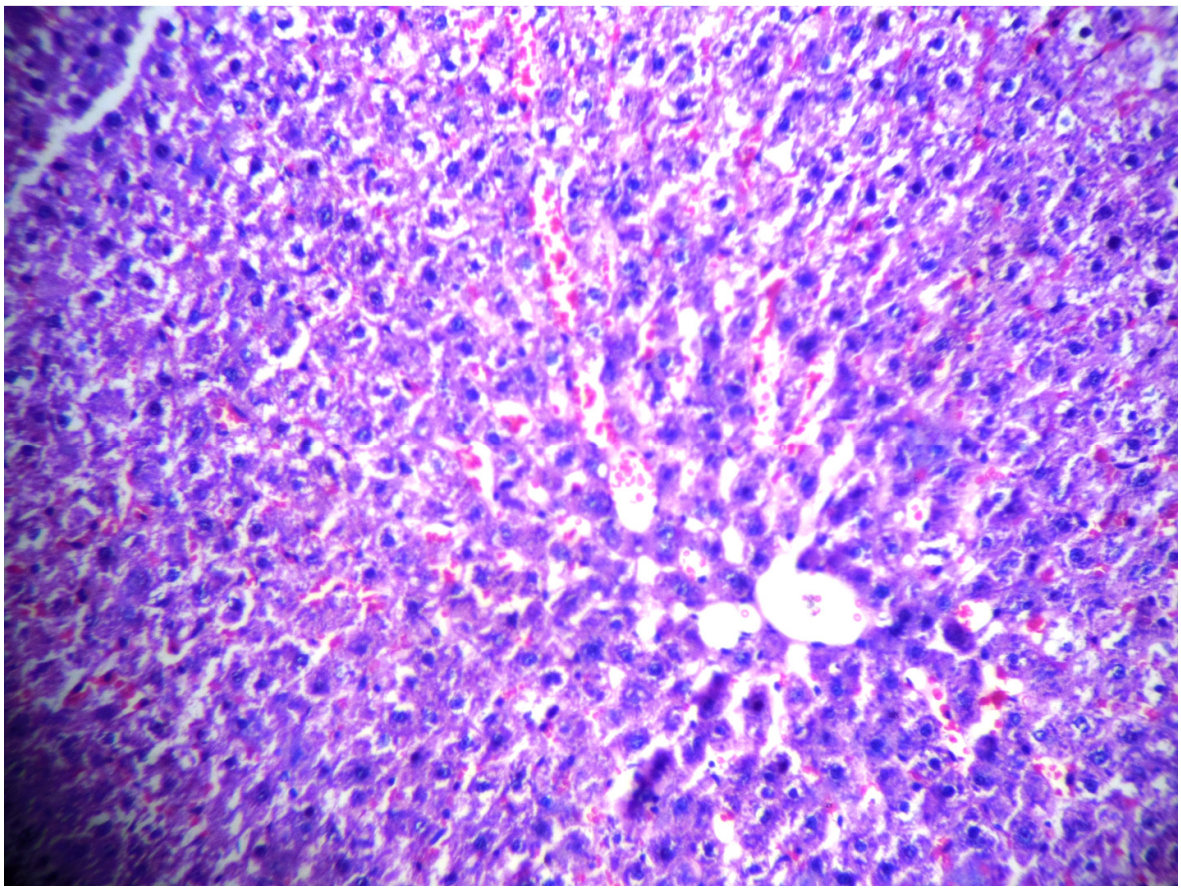

Fig.no.:9

Liver section of GP_2 (toxic control)

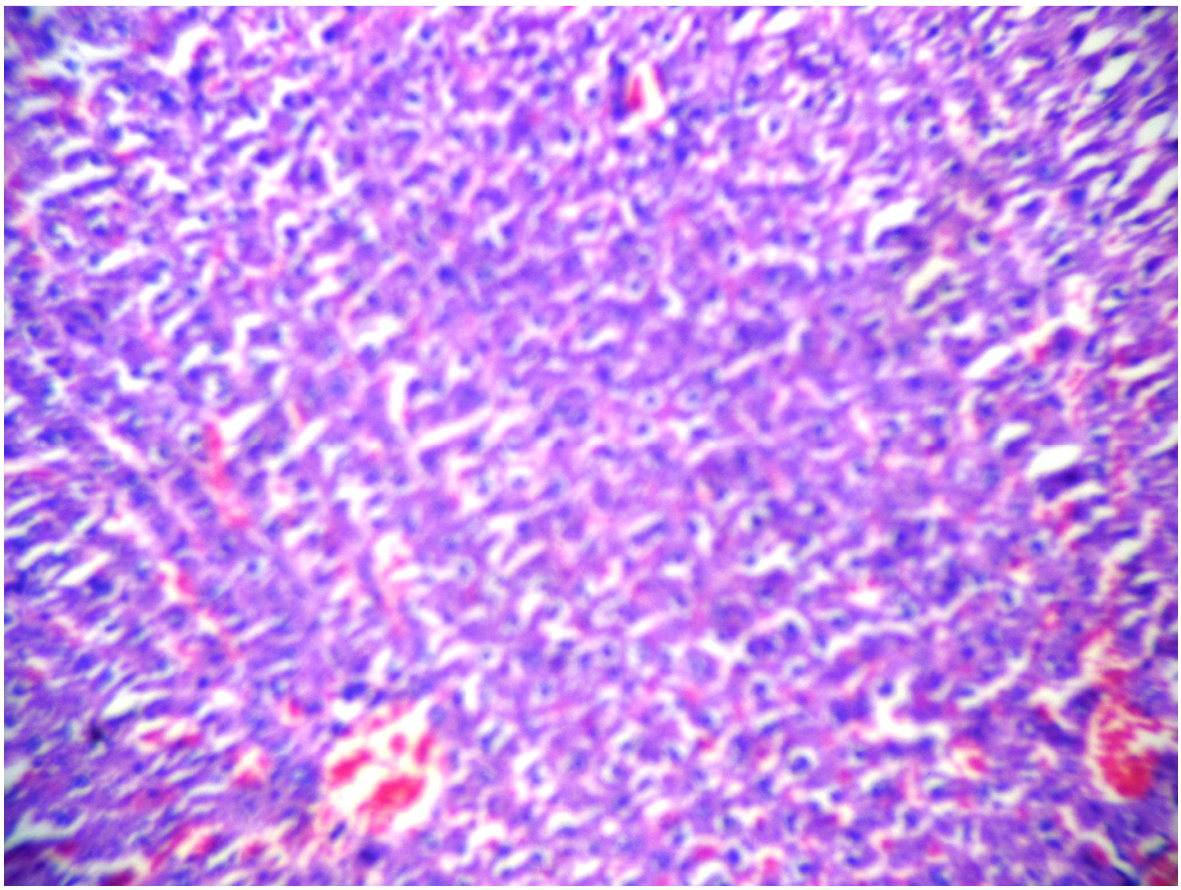

Fig.no.:10

Liver section of GP_3 (standard control)

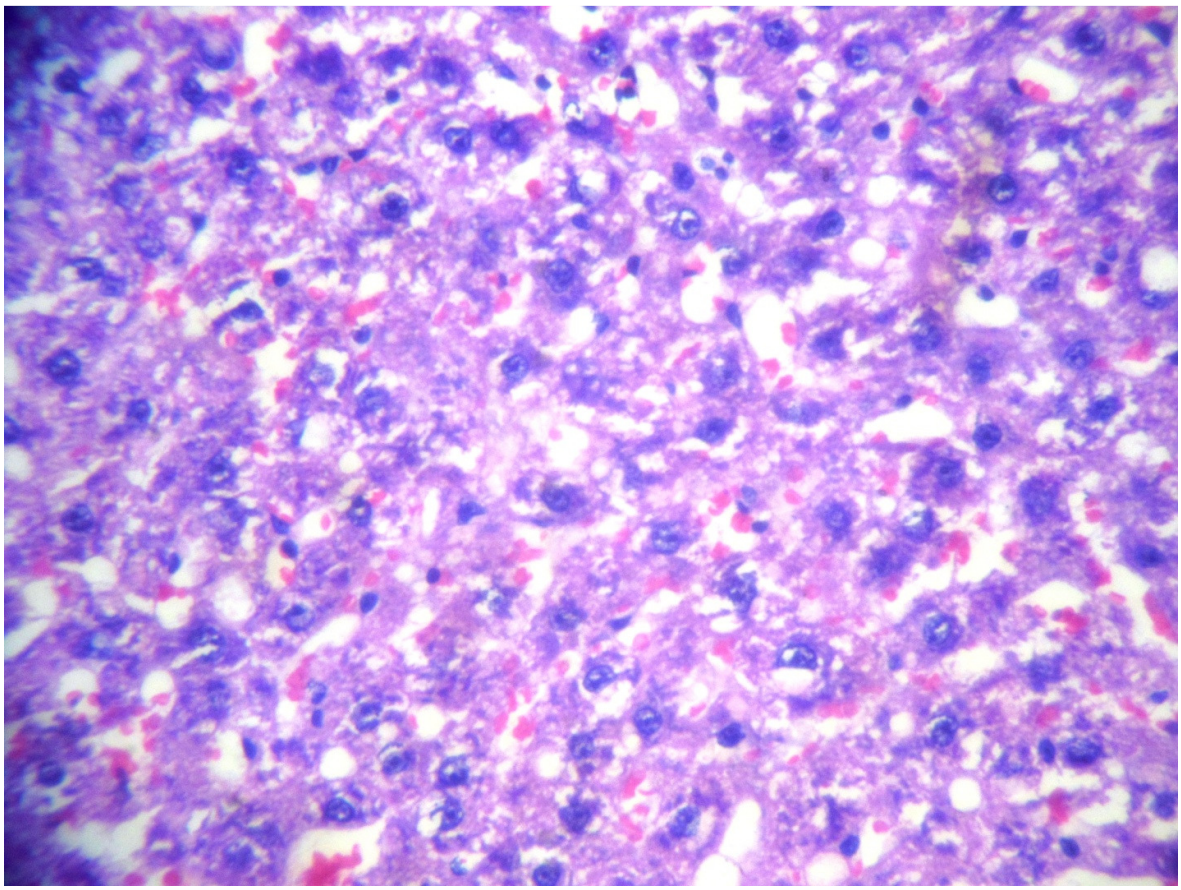

Fig.no.11

Liver section of GP_4 (*Cnidoscolusphyllanthus*200 mg/kg/rat)

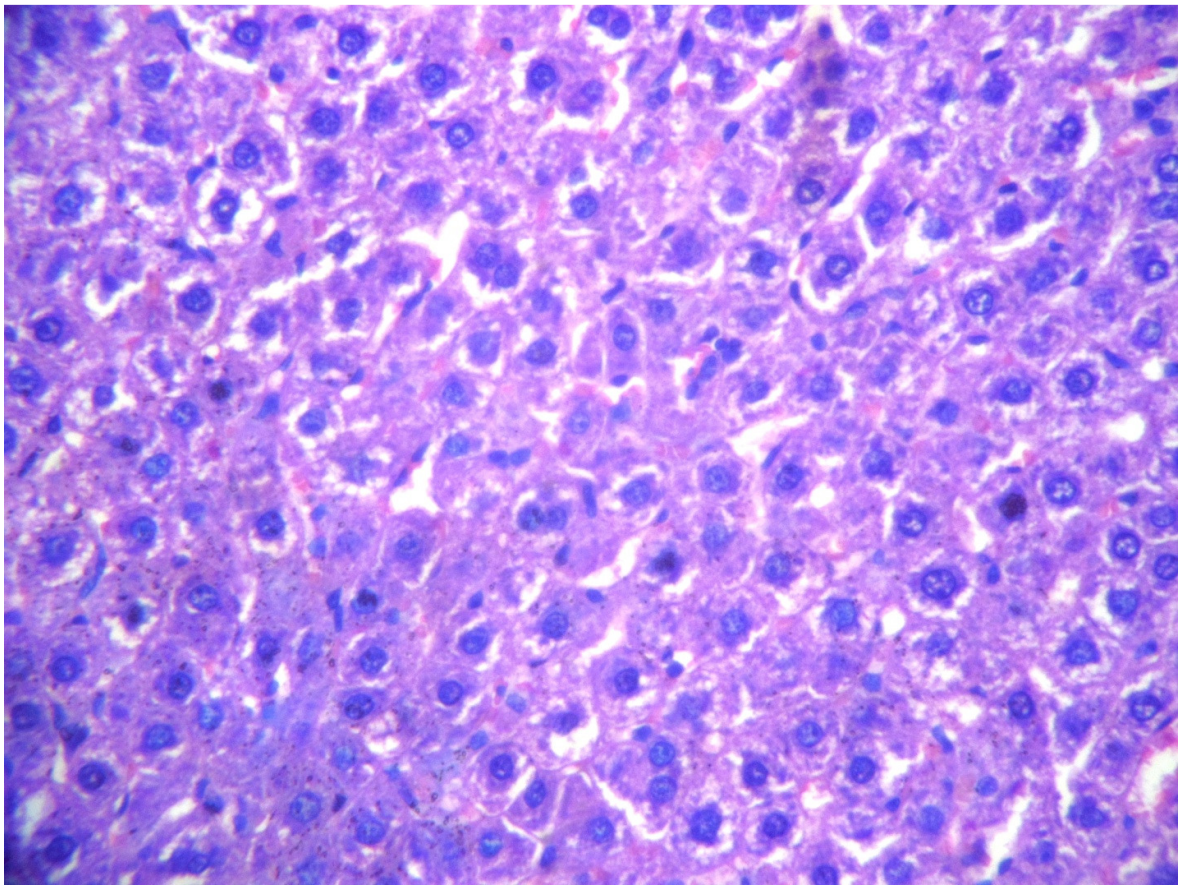

Fig.no.12

Liver section of GP_5 (*Cnidoscolusphyllanthus*400 mg/kg/rat)

RESULT

BIOCHEMICAL OBSERVATIONS

Significant increase in (P< 0 .01) Serum Aspartate Transaminase (AST) , Alanine Transaminase (ALT) , Alkaline phosphatase (ALP) , Total bilirubin (TB) and Gamma-glutamyl transpeptidase(GGTP) and significant decrease in (P< 0.01) Total protein(TP) and Total albumin(TA) levels were observed in animals treated with galactosamine 25mg/kg (Group II) as compared to normal control group(Group I).

Pretreatment with Ethanolic extract of *Cnidoscolus phyllanthus* (EECP) at a dose 200mg and 400mg /kg ,orally for 21days decreased the levels of above indices like AST ,ALT , ALP, TB, GGTP and increased levels of TP and TA significantly(P <0.01)in group IV and V.

Vitamin-E pretreatment produced significant decrease in (P< 0.01) serum AST, ALT, ALP, TB,GGTP and significant increase in TP and TA at (P< 0.01) in group III.

BIOCHEMICAL OBSERVATION IN LIVER HOMOGENATE TISSUE

In liver homogenate, there was significant decrease in SOD, CAT and GPx levels and increase in LPO levels were observed in animals treated with galactosamine 25mg/kg (group II) as compared to normal control group (Group I).

Pretreatment with Ethanolic extract of *Cnidoscolus phyllanthus* (EECP) at a dose of 200mg/kg and 400mg/kg orally for 21 days increase the levels of above indices like SOD,CAT and GPx levels and decrease levels of LPO significantly (P<0.01) in group IV and V.

Vitamin-E pretreatment produced significant increase in (P< 0.01) Liver homogenate enzyme such as SOD, CAT, GPx levels and decrease the levels of LPO significantly (P<0.01) in group III.

Table no shows the levels of non-enzymatic antioxidants such as reduced glutathione, Vitamin C and Vitamin E in the tissues (liver) of D-galactosamine hepatotoxic and control rats. The levels of non-enzymatic antioxidants in D-galactosamine hepatotoxic rats

significantly decreased. EECP both doses administered rats showed significantly increased levels of these non-enzymic antioxidants as compared with untreated hepatotoxic rats.

HISTO PATHOLOGICAL OBSERVATIONS

Histology of liver sections of normal control animals (Group I) showed normal liver architecture with were brought out central vein, were preserved cytoplasm and prominent nucleus and nucleolus (Fig no:8). The liver sections of galactosamine treated animals (Group II) showed hepatic cells with serum toxicity characterized by inflammatory cell collection, scattered inflammation across liver parenchyma, focal necrosis and swelling up of vascular endothelial cells (Fig no:9).

Vitamin-E (Group-III) exhibited protection from galactosamine induced changes in the liver (Fig no:10).

Ethanolic extract of *Cnidoscolus phyllanthus* (EECP) pretreatment at a dose of 200mg and 400mg/kg (group IV and V) appeared to significantly prevent the galactosamine toxicity as revealed by the hepatic cells with were preserved cytoplasms. EECP pretreatment also caused marked decrease in inflammatory cells (Fig no: 11 and 12).

DISCUSSION

D-galactosamine is a well-established hepatotoxicant that induces a diffuse type of liver injury closely resembling human viral hepatitis[80]. Liver damage induced by D-galactosamine, reflects disturbances of liver cell metabolism, which lead to characteristicchanges in the serum enzyme activities. Elevated serum enzymes are indicative of cellular leakage and loss of functional integrity of the hepatocyte[81].When the liver cell plasma membrane is damaged, a variety of enzymes such as aspartate aminotransferase,alanine aminotransferase, alkaline phosphatase ,total bilurubin and gamma-glutamyl transpeptidase are released into the blood stream. Their estimation in the serum is useful as a quantitative marker of the extent and type of hepatocellular damage.

In D-galactosamine induced toxicity, increased activities of aspartate aminotransferase, alanine aminotransferase,alkaline phosphatase,total biluribin and gamma-glutamyl transpeptidase and decrease activities of total protein and total albumin were observed in serum. EECP seems to preserve the structural integrity of the hepatocyte membrane as evidenced from the significant reduction in the activities of these enzymes. The 400mg/kg dose had a better effect than the low dose of EECP (200mg/kg). The higher concentration might have resulted in the production of more by products that would have interfered with the activity. Treatment with EECP significantly decreased these enzyme activities, indicating that EECP has a hepatoprotective effect against a D-galactosamine-induced liver injury.

D-galactosamine-induced oxidative damage is generally attributed to the formation of the highly reactive hydroxyl radical (OH·), the stimulator of lipid peroxidation and the source of destruction and damage to the cell membrane[82]. D-galactosamine toxicity enhanced lipid peroxidation and reduced antioxidants were reported in the kidney.[83] The previous studies show that D-galactosamine-induced rats significantly increased thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes in liver and kidney[84,85]. In the present study, we observed an increase in the levels of thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes in the tissues of D-galactosamine-hepatotoxic rats. Increased lipidperoxidation in various tissues has long been known to cause functional degradation; thus, the degradation of vital tissue leading to complications may be indirectly due to increased oxidative stress.

Treatment with EECP and Vitamin-E showed a significant reduction which might be due to the antioxidant ability of these compounds and the consequent reduction in lipid peroxidation. EECP possesses antioxidative and free-radical scavenging effects.

Oxidative stress is an imbalance between reactive oxygen species and the antioxidant defense mechanisms of a cell or tissue, which leads to lipid peroxidation, DNA damage, and the inactivation of many enzymes[86]. The enzymatic antioxidant defense system is the natural protector against lipid peroxidation that includes superoxide dismutase, catalase and glutathione peroxidase.Reduced activities of these enzymes in the tissue of D-galactosamine-hepatotoxic rats were observed in our study. Superoxide dismutase protects against the superoxide radical (O2·−), which damages the membrane and its biological structure. Catalaseprimarily decomposes hydrogen peroxide to H_2O at a much faster rate, sharing this function with glutathione peroxidase. Glutathione peroxidase may play an important role in the removal of lipid hydroperoxides. The balance between these enzymes is important for the efficient removal of oxygen radicals from tissues [87]. Therefore, reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and H_2O_2. Significant increases in the activities of these enzymes were observed on EECP administration.

The second line of defense consists of the non-enzymic scavengers glutathione, ascorbic acid, and α-tocopherol, which scavenge residual free radicals escaping from decomposition by the antioxidant enzymes. Moreover, enzymic antioxidants are inactivated by the excessive levels of free radicals and hence the presence of non-enzymic antioxidants is presumably essential for the removal of these radicals [88].Glutathione a major non-protein thiol in living organisms plays a central role in coordinating the antioxidant defense process. Glutathione reacts directly with reactive oxygen species and electrophilic metabolites, protects the essential thiol group from oxidation, and serves as a substrate for several enzymes including glutathione peroxidase. The lowered glutathione in D-galactosamine induced rats represents the increased utilization of glutathione as a result of oxidative stress.Perturbation in the redox status of glutathione not only impairs cellular defense against toxic compounds but also results in enhanced oxidative stress and oxidative injury [89]. Apart from glutathione, α-tocopherol and ascorbic acids are important free-radical scavengers which protect cell membrane against toxic agents. Both vitamins C and E have a synergistic action in scavenging oxygen-derived free radicals[90].Vitamin C functions as a free-radical

scavenger of oxygen radicals and successfully prevents detectable oxidative damage under all types of oxidative stress. Ascorbic acid appears to trap the peroxyl radical in the aqueous phase with a rate large enough to lipids and dehydroascorbate is produced in this reaction. A thiol cycle converts the dehydroascorbate into ascorbate. The thiol cycle consists of a GSSG/GSH couple[91]. Thus glutathione in blood keeps up the cellular levels of the active form of vitamin C. When there is a reduction in glutathione, the cellular level of ascorbic acid is also lowered. The observed decrease in the levels of α-btocopherol and ascorbic acid in the D-galactosamine rats might be due to an antioxidant defense against increased ROS or due to a decrease in glutathione levels in D-galactosamine-hepatotoxic rats[92]. In this respect, reported that ascorbic acid and α-tocopherol decreased in liver diseases, particularly in D-galactosamine- hepatotoxic rats. Our study observed increase the levels of these antioxidants in EECP and Vitamin-E administered rats.

The ability of EECP to enhance the levels of antioxidants along with its antilipid peroxidativeactivity suggests that this compound might be potentially useful in counteracting free-radical-mediated tissue damage caused by hepatotoxicity. Studies on the antioxidative potency of various flavonoids have confirmed the importance of the distribution and quantity of the hydroxyl groups. In general, the antioxidative properties of polyphenolsdepend on hydroxylation of ring B. The present results corroborate the protective action of EECP in D-galactosamine intoxication of rats, particularly noticeable with the high dose used by us (400 mg/kg body weight). Supplementation with this flavonoid ameliorated thehepatoprotective and antioxidant activity in D-galactosamine-induced hepatitis in rats.

CONCLUSION

In conclusion, our findings demonstrated that EECP at both doses possesseshepatoprotective and antioxidant activity, which is evidenced by lowered serum hepatic marker enzyme activities. Among the two dosages tested,400 mg/kg/body weight showed more promisinghepatoprotective and antioxidant activity, and is comparable to the standard drug Vitamin-E.

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Errata

Page no.	Printing Error	Correction